Testicular germ cell tumors : assessing the impact of occupational and environmental exposure to pesticides

Rémi Béranger

Numéro d'ordre: 309-2014 Année 2014

THESE DE L'UNIVERSITE DE LYON

Délivrée par
L'UNIVERSITE CLAUDE BERNARD – LYON 1
Ecole Doctorale Interdisciplinaire Science et Santé

Pour l'obtention du
DIPLOME DE DOCTORAT
Mention « Epidémiologie, Santé publique, Recherche sur les services de santé »

Présentée par
Rémi BERANGER

Tumeurs germinales du testicule : Etudier l'impact des expositions professionnelles et environnementales aux pesticides

Testicular germ cell tumors: Assessing the impact of occupational and environmental exposure to pesticides

Thèse dirigée par Béatrice FERVERS et Joachim SCHÜZ

Soutenue publiquement le 10 Décembre 2014
devant le jury composé de:

M. Pierre LEBAILLY (rapporteur)
M. Roel VERMEULEN (rapporteur)
M. Andreas STANG (examinateur)
M. Bruno COMBOURIEU (examinateur)
M. Vincent BONNETERRE (examinateur)
Mme. Béatrice FERVERS (directeur de thèse)
M. Joachim SCHÜZ (directeur de thèse)

Tumeurs germinales du testicule : Etudier l'impact des expositions professionnelles et environnementales aux pesticides

Les tumeurs germinales du testicule (TGCT) sont la forme de cancer la plus fréquente chez les hommes jeunes (15-39 ans). Un rôle de l'environnement au moment de la période prénatale est suspecté, mais aucune étiologie claire ne semble émerger. Cette thèse avait pour but de développer une nouvelle approche épidémiologique pour étudier l'impact des expositions prénatales aux pesticides sur le risque de TGCT.

Par une revue de la littérature, nous avons tout d'abord montré le manque d'études sur les expositions prénatales et le besoin de méthodes fiables pour évaluer l'exposition environnementale aux pesticides. Ensuite, par une campagne de mesures domestiques dans 239 foyers, nous avons identifié les déterminants environnementaux de l'exposition aux pesticides agricoles. La surface des cultures dans un rayon de 500m (vergers) ou 1000m (céréales/vignes), le vent et les barrières végétales ont été identifiés comme déterminants de l'exposition. La bonne efficacité de notre lingette en cellulose a été testée en laboratoire. Nos résultats montrent également l'importance des utilisations domestiques de pesticides sur la contamination des foyers. Enfin, à travers une étude cas-témoins pilote, nous avons confirmé notre capacité à recruter des sujets et leurs mères, ainsi que les informations requises pour évaluer les expositions jusque dans les années 70.

Pour conclure, nos résultats ont permis le développement d'une étude cas-témoins nationale (projet TESTIS) pour étudier l'impact des expositions prénatales aux pesticides sur le risque de TGCT. Ce projet a été financé et est en cours de réalisation. Cette thèse sert également de base à plusieurs autres projets multidisciplinaires.

Mots clés : Tumeurs germinales du testicule ; Pesticides ; Système d'information géographique ; Expositions environnementales ; Epidémiologie ; Revue de la littérature ; Etude de validation.

Testicular germ cell tumors: Assessing the impact of occupational and environmental exposure to pesticides

Testicular germ cell tumors (TGCT) are the most common cancers in men aged 15–39 years. Environmental exposures occurring in the prenatal period are suspected to play a role, but no clear associations with TGCT risk are known. This thesis aimed to develop an epidemiological approach to study the impact of prenatal exposures to pesticides on the TGCT risk.

First, through a systematic literature review, we identified a gap in knowledge regarding prenatal exposures, as well as the need for more reliable assessment of environmental pesticide exposures. Second, through a survey of indoor dust sampling in 239 households, we identified the environmental determinants of agricultural pesticide exposure to develop a metric to assess environmental pesticide exposures using a geographical information system. Crop acreage within 500m (orchards) or 1000m (cereals/vineyards), wind, and vegetative barriers were identified as determinants of the indoor contamination. The overall good efficiency of our cellulose wipe was assessed through laboratory experiments. Our results also suggested domestic pesticide use as a major source of households' pesticide exposure. Third, through a case-control pilot study we tested different approach to recruit young men and their mothers, and we confirmed our ability to collect information about their exposures, and to map precisely their addresses until the 1970's.

Our findings lead to the development of a national case-control study (TESTIS project) aiming to assess the impact of prenatal pesticides exposures on the TGCT risk. This project has been funded and is currently on-going. Our research also serves as basis for further multidisciplinary projects.

Keywords: Testicular Neoplasms; Pesticides; Geographic information systems; Environmental exposures; Epidemiology; Literature review; Validation study.

Author's affiliations

The work presented in this thesis has been done in the following units:

- Unité cancer et Environnement
  Centre Léon Bérard
  28 Rue Laennec, 69373 Lyon 08 Cedex

- Section of Environment and Radiation,
  International Agency for Research on Cancer,
  150 rue Albert Thomas, 69372 Lyon 08 Cedex

- EAM 4128 "Santé Individu Société"
  Université Claude Bernard – Lyon 1
  43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex

Acknowledgements

I would like to acknowledge Béatrice Fervers and Joachim Schüz, my thesis supervisors, for their teaching, their kindness, their open-mind, and for letting me the chance to prove myself.

I am grateful to the reviewers (Roel Vermeulen and Pierre Lebailly), as well as the jury members (Andreas Stang, Bruno Combourieu, and Vincent Bonneterre) for their time and to have shared their expertise with me.

I also would like to acknowledge all my financial supports: the Rhone-Alpes region (for my doctoral grant and for the SIGEXPO project); the *Fondation de France* (pour the TESTEPERA and the SIGEXPO projects); the Anses (for the TESTEPERA project); the INCa (for the TESTEPERA and TESTIS projects); the INSERM (for the TESTIS project); the CLARA (for my mobility grant to the US National Cancer Institute and for the TESTEPERA project); the LYRIC (for supporting the collaborative program between CLB and IARC on "Pesticides and Cancer").

I am proud and pleased to have worked with Jeffrey, Elodie, Charlotte, Olivia, and Elise during these three years. Thanks to them and to my other colleagues from the Cancer and Environment unit, and the Environment and Radiation section (by alphabetic order to avoid endless conflicts), who greatly contribute to the achievement of this thesis: Ann, Anya, Aude-Marie, Audrey, Aurélie, Ausra, Blandine, Carolina, Caroline, Catarina, Catherine, Cedric, Christine, Claire, Etienne, Friederike, Ghassan, Gilles, Guillaume, Helen (my personal English spell-checker), Isabelle D & Isabelle TC, Jelle, Joane, Julien, Kévin (the worst driver I ever seen), Maria, Marie-Laure, Marina, Marine, Mary-Louise (the bush telegraph), Monica, Nicole, Laure, Leah, Lucian, Rachel D & Rachel H, Renaud, Sara, Simon, Thomas, Valerie, Thierry (especially for his office), Veronique… (sorry for those I had forgotten).

Finally, I would like to warmly thank my family and my friends for their support and their patience.

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List of abbreviations (alphabetic order)

AEI: agricultural exposure index

AFSSET: *Agence Française de Sécurité Sanitaire de l'Environnement et du Travail* (French agency for environmental and occupational safety)

ART: center for assisted reproductive technology

BRC: biological resource center

CAP: Contributive area for pesticide drifts

CCTIRS: *Comité Consultatif sur le Traitement de l'Information en matière de Recherche dans le domaine de la Santé* (French ethical committee)

CECOS: *Centres d'étude et de conservation d'œufs et de sperme humain* (center for treatment of infertility)

CNIL: *Commission Nationale de l'Informatique et des Libertés* (French regulatory authority)

CLARA: Cancéropole Lyon Auvergne Rhône-Alpes

CLB: Centre Léon Bérard

CLCC: *Centre de Lutte Contre le Cancer* (French cancer centers)

DAC: departmental agricultural chamber

db-RDA: distance-based redundancy analyses

DDE: dichlorodiphenyldichloroethylene

DDAAF: *Direction Départementale de l'Alimentation, de l'Agriculture et de la Forêt* (departmental directorate for food, agriculture and forestry)

DDT: dichlorodiphenyltrichloroethane

DGDDI: *Direction Générale des Douanes et des Droits Indirects* (Directorate-General of Customs and Indirect Taxes)

DMF: dimethylformamide

DNA: deoxyribonucleic acid

DNOC: dinitro-*ortho*-cresol

DRAAF: *Direction Régionale de l'Alimentation, de l'Agriculture et de la Forêt* (Regional directorate for food, agriculture and forestry)

ECA: effective contributive area for pesticide drifts

EDI: european deprivation index

EDTA: ethylene diamine tetra-acetic acid

GC: gaz chromatography

GIS: geographical information system

GPS: global positioning system

HCB: hexachlorobenzene

HPLC: high performance liquid chromatography

HVS3: high volume small surface sampler

IARC: International Agency for Research on Cancer

IFEN: *Institut Français de l'Environnement* (French institute of environment)

IGN: *Institut National Géographique* (French national geographic institute)

INCa: *Institut National du Cancer* (French national cancer institute)

INSEE: *Institut National de la Statistique et des Etudes Economiques* (French institute for statistics and economic studies)

INSERM: *Institut National de la Santé et de la Recherche Médicale* (French national institute of health and medical research)

InVS: *Institut de Veille Sanitaire* (French Institute for public health surveillance)

IQR: inter-quartile range

IRIS: aggregated units for statistical information

ISCO: international standard classification of occupations

JEM: job-exposure matrix

Koa: octanol-air partition coefficient

Koc: octanol-carbon partition coefficient

Kow: octanol-water partition coefficient

LQ: limit of quantification

MS: mass spectrometeur

MRM: multiple reaction monitoring

NAF: *Nomenclature d'activité française* (French nomenclature of activity)

NOS: Newcastle-Ottawa Quality Assessment scale

NS: non-significant

ODS: old dust sample

PB: piperonil butoxide

PBDE: polybrominated diphenyl ether

PCA: principal component analysis

PCB: polychlorinated biphenyl

PCoA: principal coordinate analysis

PEA: pesticide exposures during adolescence

PEPPP: pesticide exposures during prenatal and early postnatal period

PI: principal investigator

PVC: polyvinyl chloride

RDA: redundancy analyses

RDS: recent dust samples

RPG: *Registre Parcellaire Graphique*

RSD : relative standard deviation

SB: structural barriers

SES: socio-economic status

SDR: surface dislogeable residues

SIG: see GIS

SL: surface loading

SNP: single Nucleotide Polymorphism

SRM: standard reference material

SVOC: semi-volatile organic compounds

TB: Topographic barriers

TGCT: testicular germ cell tumor

TDS: testicular dysgenesis syndrome

USEPA: United States Environmental Protection Agency

VB: vegetative barriers

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Chapter I:
General introduction

I.1 Synthèse en français / summary in English

SYNTHESE - FRANCAIS

Les tumeurs germinales du testicule (TGCT) représentent la forme de cancer la plus fréquente chez les hommes âgés de 15 à 39 ans, et l'incidence de cette pathologie augmente régulièrement depuis les 30 dernières années. Les hypothèses de recherche actuelles s'orientent vers le rôle des facteurs environnementaux survenant pendant la période prénatale. Malgré de nombreuses études portant sur les expositions professionnelles et environnementales, aucune étiologie ne semble clairement émerger. Toutefois, si de multiples études se sont intéressées aux pesticides, les méthodes utilisées pour caractériser les expositions restent limitées, et très peu d'étude se sont intéressées aux expositions prénatales.

Des travaux précédents ont montrés que les foyers les plus proches des cultures sont plus exposés aux pesticides agricoles. Sur ce principe, les systèmes d'information géographique (GIS) sont considérés comme une méthode pertinente pour évaluer les expositions environnementales aux pesticides agricoles. Toutefois, des travaux de validation étaient nécessaires pour pouvoir utiliser une approche GIS en France dans ce contexte. Les prélèvements de poussières domestiques ont été présentés comme une méthode pertinente pour estimer l'exposition d'un ménage aux pesticides et peuvent servir de bases pour la validation d'une métrique pour GIS.

Pour clarifier les hypothèses actuelles concernant les TGCT, cette thèse avait pour but de développer une approche épidémiologique permettant d'étudier l'impact des expositions prénatales aux pesticides sur le risque de développer une TGCT. Les étapes nécessaires à la réalisation de la thèse étaient : 1/ l'identification précise des carences de la littérature concernant le lien entre pesticides et TGCT, à travers une revue de la littérature (**Chapitre II**) ; 2/ le développement d'une nouvelle métrique GIS pour évaluer les expositions environnementales aux pesticides de manière plus fiable (**Chapitre III**) ; 3/ la réalisation d'une étude cas-témoins pilote pour optimiser le design de l'étude finale et pour vérifier notre capacité à recueillir les expositions jusque dans les années 70

(**Chapitre IV**) ; 4/ le développement d'une étude cas-témoins répondant aux objectifs de la thèse, en tenant compte des étapes précédentes (**Chapitre IV**)

SUMMARY - ENGLISH

Testicular germ cell tumors (TGCT) are the most common cancer in men aged 15 to 39 years. TGCT incidence has increased steeply over the past 30 years. Current etiologic hypotheses suggest that TGCTs are related to environmental exposures occurring in the prenatal period. Specifically, exposures to anti-androgenic endocrine disruptors are plausible risk factors, but numerous studies of such exposures in environmental and occupational settings have not found a clear link with TGCT. This is also the case for pesticide exposures in relation to TGCT. However, exposure assessment methodology used was generally crude, and few studies specifically focused on prenatal exposures.

Previous studies have shown that households located proximate to agricultural fields had higher levels of agricultural pesticides. Based on this relationship, Geographical Information Systems (GIS) have been suggested as an efficient tool to retrospectively assess environmental exposures to agricultural pesticides. However, no GIS-based pesticide exposure metric has been developed for or validated in France. Measurements of pesticides in indoor dust as estimates of indoor pesticide contamination serve as an efficient approach to develop such a GIS-metric.

To address current hypotheses on TGCT etiology, this thesis aimed to develop an epidemiological approach to assess the relationship between prenatal pesticide exposure and risk of TGCT. The specific research objectives of the thesis were: 1/ to identify more precisely gaps in knowledge on environmental and occupational risk factors of TGCT, through the conduct of a literature review (**chapter II**); 2/ to develop GIS metrics to assess environmental pesticide exposures more reliably (**chapter III**); 3/ to conduct a pilot case-control study to optimize the study design for future implementation in France, and to examine the feasibility of estimating exposures dating back to the 1970's (**chapter IV**); 4/ to design a case-control study to be conducted in France in accordance with the

aim of the thesis and informed by findings from the other objectives of the thesis (**chapter IV**).

I.2 Scientific background

I.2.1 Testicular germ cell tumors in young men

a) Epidemiology

Testicular cancers represent the most frequent cancer in young men aged 15 to 39 years in developed country with primarily Caucasian population (Forman et al. 2013). Testicular cancers have been increasing over the last decades in industrialized countries, with the highest rates in Europe and US (Chia et al. 2010b). In France, the annual incident rate has increased from 3.4/100,000 in 1980 to 6.7/100,000 in 2008 (Belot et al. 2008). Similar trends have been observed in the rest of Europe with an increase in incidence of 153% in Germany, 131% in Finland and 116% in Norway, over the past 30 years (Chia et al. 2010b). However incidence rates vary substantially from one country to another, with a southwest-northeast gradient across the European continent (Adami et al. 1994; Purdue et al. 2005). The burden is predicted to rise by 24% by 2025 in Europe, but strong discrepancies in incidence would remain between countries (Le Cornet et al. 2014).

Testicular germ cell tumors (TGCT) represent 98% of the testicular cancers (Forman et al. 2013). TGCT regroups two main histologies in the young men: seminomas, that peak around 35 years of age, and non-seminomas, that peak around 25 years of age. TGCTs in young adults should be distinguished from other rarer TGCTs histologies, which have different pathogenesis: yolk sac tumors and immature teratomas occurring during childhood, and spermatocytic seminoma affecting mostly men over 50 years of age (Eble et al. 2004; Rajpert-De Meyts 2006). TGCT have good prognostic, more than 95% of survival at five years for localized tumors and about 80% when metastatic (Feldman et al. 2008).

b) Known or suspected etiologies

Environmental factors are strongly suspected to be related to TGCT, considering the important geographical and temporal variations in incidence rates. The evolution of the incidence rate in migrant populations, between the first and the second generation, also supports this hypothesis (Hemminki and Li 2002; Myrup et al. 2008; Schmiedel et al. 2010). Given the peak incidence of TGCT in young adults and the fact that TGCT have been shown to developed through carcinoma-in-situ cells of fetal origin (Rajpert-De Meyts 2006), the role of early exposures, in particular intra-uterine, have been hypothesized (INSERM 2008; Skakkebaek et al. 2001).

In 2001, Skakkebaek et al. (2001) suggested that TGCT, cryptorchisim, hypospadias and several forms of infertility should be part a common underlying disorder occurring during the fetal life: the Testicular Dysgenesis Syndrome (TDS). This hypothesis is supported by the strong association between TGCT and cryptorchidism, the young age of TGCT patients, and simultaneous decreasing trends in semen quality and increasing trends in TGCT. TDS could be due to a malfunction of the Leydig and/or Sertoli cells during the development of the testis, probably induced by altered production/action of the testosterone (Sharpe and Skakkebaek 2008). Endocrine disruptors, especially estrogenic and anti-androgenic compounds, may play a role in the formation of some of the TDS forms, especially in individuals with genetic susceptibility (Dalgaard et al. 2012). The window of exposure should be during the 1st trimester of the pregnancy in humans, based on extrapolation from animal experimentations (Welsh et al. 2008). However, since the hypothesis of a prenatal origin of TGCT and the role of environmental factors remain commonly admitted, the concept of TDS remains controversial (Akre and Richiardi 2009; Joffe 2011).

Numerous individual risk factors such as size and weight at birth, birth order, pre-term birth, month of birth, mothers' use of hormones during pregnancy (including the diethylstilbestrol), tobacco and marijuana consumption, body mass index, testis traumatisms as well as physical activity have been studied (Cook et al. 2009; Garner et al.

2005; McGlynn and Cook 2009). However, none of these have been shown to be a strong determinant of TGCT and these associations are not likely to explain the rise of the TGCT during the previous decades. Several studies have also suggested a positive association between higher socio-economic status and the occurrence of TGCT (Pukkala and Weiderpass 2002; Swerdlow et al. 1991; Swerdlow and Skeet 1988; Van den Eeden et al. 1991). However, this relation is not consistently found (Sarfati et al. 2011).

Genetic factors have been pointed out since TGCT risk varies by ethnicity (Caucasian men have higher risk than Asian and African men) (Bray et al. 2006). Familial history of TGCT is also known to be associated with increased TGCT risk and it is estimated that 13% of TGCT are of genetic origin (Dalgaard et al. 2012). Several polymorphisms associated to KIT-Ligand and the TERT complexes have been identified to be associated with TGCT risk (Dalgaard et al. 2012; Kanetsky et al. 2009; Kratz et al. 2011; Rapley et al. 2009; Turnbull et al. 2010). Furthermore, there are suggestions that some polymorphisms involved in persistent organic pollutant metabolism pathways (in particular 2 loci: CYP1A1 and HSD17B4) may modify the associations between polychlorinated biphenyl (PCB) exposure and TGCT risk (Chia et al. 2010a).

Numerous environmental and occupational risk factors have been studied. Among these, pesticides were first suggested by Mills et al. in 1984 (Mills et al. 1984) and appear to be one of the most studied risk factors in the literature. Available literature reviews on adulthood and adolescent exposure have not shown a clear pattern for pesticides exposures or related occupations (Garner et al. 2008; Garner et al. 2005; McGlynn and Trabert 2012). McGlynn and Trabert (2012) suggested that firefighting, aircraft maintenance and exposure to some organochlorine compounds are likely to be associated to higher TGCT risk, but did not identify additional group of exposures at higher risk of TGCT. Additional studies have investigated the role of the place of residence (urban versus rural location), as surrogate for environmental pesticide exposures, but showed inconsistent results and none of these included the residential history (Doll 1991; Schouten et al. 1996; Sonneveld et al. 1999; Walschaerts et al. 2007). More in depth

description and discussion of identified environmental and occupational risk factors are presented in the second chapter of this thesis.

Only a few studies have investigated parental exposures despite the current hypotheses of intrauterine origin of TGCT. Kristensen et al. (1996) showed an excess risk amongst sons of farmers that used high nitrate/phosphate ratio fertilizer (suggesting intensive farming), but the role of paternal occupational pesticides exposure showed inconsistent results (Giannandrea et al. 2011; Kardaun et al. 1991; Kristensen et al. 1996; Moller 1997; Rodvall et al. 2003). Overall, these studies are limited by small sample size (Giannandrea et al. 2011; Rodvall et al. 2003) or broad exposure assessment (Kardaun et al. 1991; Kristensen et al. 1996; Moller 1997; Rodvall et al. 2003). Nori el al. (2006) showed an increased TGCT risk among adolescents having hobbies related to endocrine disruptor exposures (painting, mechanic, etc…). Two additional studies analysed organochlorine compounds in blood samples of mothers at birth or at the son's diagnosis: the first study (based on only 20 cases) failed to show an association (Cohn et al. 2010); the second study showed an association between TGCT in sons and mothers' increased serum level of hexachlorobenzene, PCBs, polybrominated diphenyl ethers (PBDEs) or chlordane (Hardell et al. 2006).

I.2.2 Retrospective assessment of environmental pesticide exposure

a) Prerequisite for environmental exposure assessment

Because occupational exposures tend to be much higher than environmental exposures (Semple 2005), first assessing occupational exposure is a prerequisite in order to interpret environmental exposures. Several tools exist to assess occupational exposures to pesticides. Direct approaches consisting of blood or environmental sampling are generally expensive and cannot be used in retrospective case-control studies. Indirect approaches comprise questionnaire based estimations, job-exposure matrixes (JEM), or expert assessment (Brouwer et al. 2014; Stewart et al. 2001; Teschke et al. 2002;

VanTongeren et al. 2002). Recent studies have shown that occupational exposure to pesticides concern not only farmers and pesticide applicators, but also agricultural workers that enter into the fields after pesticide applications (Baldi et al. 2014), or worker involved in other jobs related to pesticide utilizations (e.g. carpenter, sawmill worker…) (Provost et al. 2007).

In addition to being exposed themselves, workers having occupational exposure to pesticides tend to contaminate their own households. Lu et al. (2000) showed that children living with parents working with agricultural pesticides have higher exposure to pesticides, based on urine spot samples and hand wiping of 109 children. Simcox et al. (1995) showed that four organophosphorous pesticides were found at higher concentration in soil and carpet dust of 26 farming and 22 farmworker families, compared to 11 non-farming reference families, in the state of Washington, US.

b) Estimating environmental pesticide exposure using geographical information systems

Retrospective exposure assessment of pesticide exposures is challenging, especially for environmental exposures since subjects are not aware of their exposure. Substantial proportions of pesticides applied on crops, up to 85% in some studies performed in the 1980's, were dispersed into the air, surface water or soil (Chester and Ward 1984). Recent studies have demonstrated that proximity to agricultural areas was associated to higher environmental pesticide exposure. Ward et al. (2006) suggested that corn and soybean field acreage within 750m from households was a significant predictor of the herbicide level in carpet dust. Similarly, Gunier et al. (2011) showed that crop acreage within 500m and 1,250m was significantly correlated to the pesticide contamination in indoor carpet dust, for five of the seven pesticides studies (stronger correlation was found using the 1,250m radius). Chevrier et al. (2014) found higher level of dealkylated triazine metabolites in urine of pregnant women living close to corn crops. Lastly, Weppner et al.

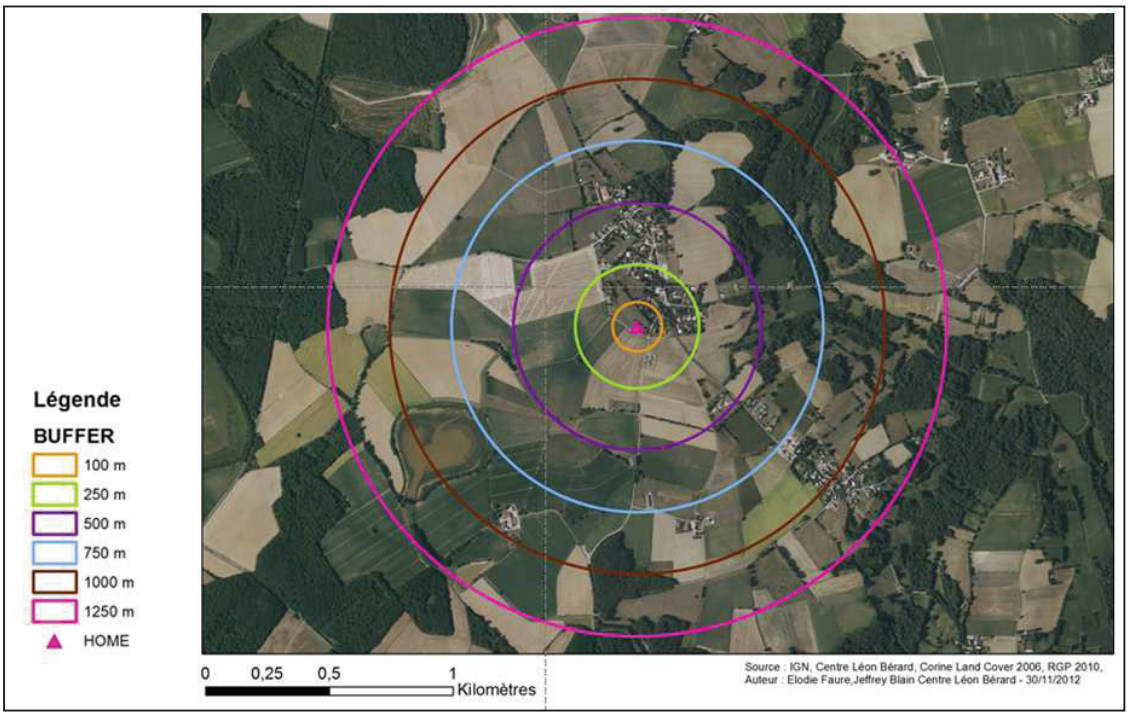
(2006) detected residues of pesticides on play-grounds, toys and children's hands after the air spreading of pesticides in surrounding potato corn and wheat fields.

Considering crop proximity as a predictor of the environmental exposure to agricultural pesticides, geographical information systems (GIS) allow to construct exposure metrics by attributing geographical coordinate to different set of data (i.e. subjects' households and agricultural crops). GIS have been considered to be relevant for estimating retrospective environmental exposure to agricultural pesticide over large areas. Moreover, GIS should minimize misclassification bias for environmental exposure assessment, by providing more objective estimations of the exposure (Nuckols et al. 2004; Zou et al. 2009). Since the last decade, due to the increasing capacity of computers, there is growing utilization of GIS to assess the impact of environmental pesticide exposure on human diseases and disorders, such as Parkinson disease (Lee et al. 2013; Ritz and Costello 2006), cancers (Brody et al. 2004; Carozza et al. 2009; Marusek et al. 2006), birth defects (Agopian et al. 2013; Rull et al. 2006a) and autism (Roberts et al. 2007).

Geographic coordinates of subjects' living place were attributed based on Global Positioning System (GPS) measurements (geolocation) or based on postal addresses (geocoding). While geolocation allow good precision, geocoding may induce some approximations. In a previous French study based on geolocation of 2779 addresses, 80.9% of addresses were precise at 200m, 16.1% were precise between 300m and 600m, and 3% at the level of the town (Sermage-Faure et al. 2012).

Considering the crop acreage within a defined buffer (area covered by a concentric circle; see Figure 1.1) has been shown to be more efficient than considering the proximity to crops to predict the agricultural pesticide exposures (Ward et al. 2006). However, buffer size varied depending on the available studies, from 500m to 1250m. To our knowledge, no clear standard exists in the literature. Several studies have also considered the major wind direction (Brody et al. 2002; Brody et al. 2004; Chevrier et al. 2014) or the presence

of forest, but the impact of these metrics on the indoor pesticide contamination have never been validated.



The figure presents different buffer from 100m to 1250m around a French house included in the SIGEXPO project. The dot at the center of the buffers represents the house. Figure was made by Elodie Faure (Centre Léon Bérard), used with permission.

Figure 1.1: Example of buffers around a French household.

c) Using indoor dust sampling to measure households' pesticide exposures

To identify predictor of environmental pesticide exposures, or to validate GIS metrics aiming to assess environmental exposures to agricultural pesticides, field measurements are needed. Previous studies either sampled dust in domestic areas (Simcox et al. 1995; Weppner et al. 2006; Ward et al. 2006; Gunier et al. 2011) or performed biological samples among inhabitants (Chevrier et al. 2014; Lu et al. 2000). However, while biological samples reflect the true human exposure, these also introduce higher variability due to various biological parameters or other sources of exposure (e.g. environmental or occupational exposures from working places…).

Since the general population spends about 85 to 90% of their time indoor, largely at home, monitoring households' exposures was considered as a relevant method to estimate subjects' environmental exposures (Butte and Heinzow 2002; Colt et al. 2004; Lioy et al. 2002).Semi-volatile organic compounds (including pesticides) have been shown to easily bind to particles such as dust (Weschler and Nazaroff 2010). House dust is a repository of pesticides and other chemicals used indoor or transported from outside, e.g. pesticides applied on nearby agricultural fields (Mercier et al. 2011; Obendorf et al. 2006). Moreover, because of protection from degradation by sunlight, fungus, and other factors, pesticides in indoor dust are more stable than in outdoor environs (Butte and Heinzow 2002). Collecting house dust samples is a relatively cheap and straightforward method to determine indoor contamination from organic contaminants (Butte and Heinzow 2002; Lioy et al. 2002; Mercier et al. 2011).

Various methods have been used to collect indoor dust from carpet floor, including high Volume Small Surface Sampler (HVS3) (Golla et al. 2011; Quiros-Alcala et al. 2011), commercial vacuum (Harrad et al. 2009; Obendorf et al. 2006) and samples from personal vacuum bag (Colt et al. 2004; Knobeloch et al. 2012). Wipes are more commonly used on hard surfaces (Stout et al. 2009; Julien et al. 2007) and are preferred in large-scale studies for its ease of use compared to vacuum (Deziel et al. 2011; Mercier et al. 2011). Also, carpets retain pesticides over time and should reflect mainly cumulative household's exposures from larger time periods (Obendorf et al. 2006) compared to wipe sampling.

I.3 Objectives of the thesis

a) Open research questions

Based on TDS hypothesis, endocrine disruptors having anti-androgenic properties represent plausible suspects. Pesticides represent an important source of exposure of

endocrine disruptors and have been frequently suggested as a credible risk factor of TGCT, but no clear association exists. However, available etiologic studies on TGCT are mainly focused on adulthood exposure, and only sparse data are available on prenatal exposure and environmental exposure. The few studies focussing on prenatal exposures suffered from limited power and/or poor exposure assessment reliability (especially for pesticides, which represent the exposures the most frequently targeted). Overall, the role of pesticides and endocrine disruptors on the risk to develop TGCT in adulthood remain unclear. A recent publication also suggested that postnatal exposure should impact as well the risk to develop TGCT (Speaks et al. 2012), and hypothesis of a combined prenatal and later life origin have been emitted (McGlynn and Trabert 2012). However, to our knowledge, this hypothesis of a combined origin has never been explored.

Due to strong differences in reproductive mechanisms and TGCT histologies between humans and current animal models, the possibility of interspecies extrapolation is limited (Eble et al. 2004) and our knowledge on TGCT etiologies are mainly based on epidemiology. Considering the rarity of the disease, case-control design is the more relevant approach to study TGCT etiologies. Therefore, one of the main difficulties remains to reliably assess the exposures and potential confounding factors during the perinatal periods, 20 to 40 years retrospectively. Retrospective environmental exposure assessment, especially for pesticides, remains challenging and will require the development of new approaches. Based on existing literature, GIS is a seductive approach but require field validation since no model has been validated in the French context. Moreover no clear recommendation exists concerning the best buffer size and available metrics only focused on crop acreage, despite recommendation from several teams to include the wind effect and the barriers in future GIS metrics (Gunier et al. 2011; Nuckols et al. 2007; Ucar and Hall 2001).

b) Objectives

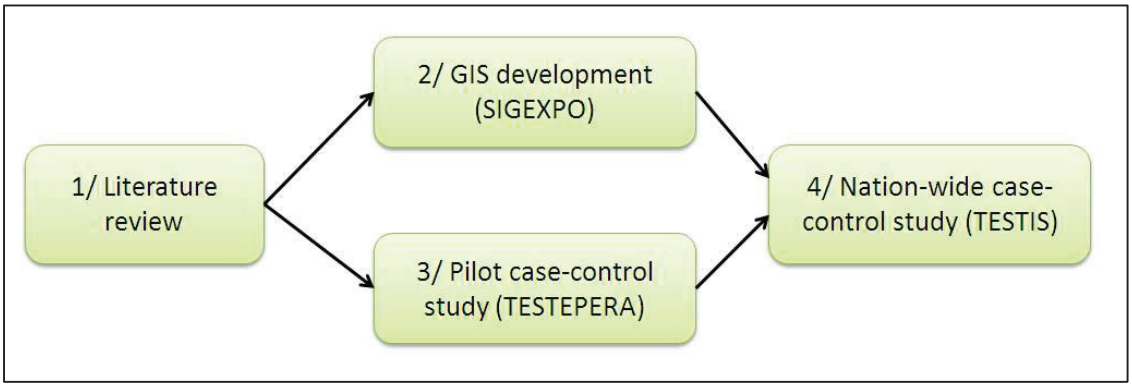
To clarify these hypotheses, the Centre Léon Bérard (France) and the International Agency for Research on Cancer (France) would like to develop a new national case-control study in France. The objective of the present thesis was to develop an epidemiological approach to assess the relation between prenatal pesticides exposures and TGCT. The overall aim of the thesis has been translated into specific research objectives (Figure 1.2):

1/ to identify more precisely gaps in knowledge on environmental and occupational risk factors of TGCT, through the conduct of a literature review (**chapter II**);

2/ to develop a GIS metric to assess environmental pesticide exposures more reliably, including retrospectively (SIGEXPO project; **Chapter III**);

3/ to conduct a pilot case-control study to optimize the study design for future implementation in France, and to examine the feasibility of estimating exposures dating back to the 1970's (TESTEPERA project; **chapter IV**)

4/ to design a case-control study to be conducted in France in accordance with the aim of the thesis and informed by findings from the other objectives of the thesis (TESTIS project; **chapter IV**)



GIS: geographical information system. The figure presents the different steps of the thesis corresponding to the different parts of the manuscript. SIGEXPO, TESTEPERA and TESTIS correspond to the different projects developed during the thesis (see chapter III, IV for more information on the projects).

Figure 1.2: Organization of the thesis.

Chapter II:
Systematic review of the literature

Identifying gaps in knowledge and clarifying the needs for future studies on testicular germ cell tumors

II.1 Synthèse en français / summary in English

SYNTHESE - FRANCAIS

Objectifs du chapitre : Alors que de nombreuses études ont étudié les TGCT, aucune étiologie claire n'a pu être mise en évidence. D'autre part, aucune revue de la littérature ne s'est intéressée aux expositions prénatales. L'objectif de notre revue systématique de la littérature était de synthétiser les connaissances concernant les facteurs environnementaux et professionnels associés au TGCT.

Revue systématique de la littérature : Nous avons identifié tous les articles publiés sur le sujet entre 1990 et 2012 par une recherche systématique sur PubMed. Les articles ont été évalué indépendamment sur le plan méthodologique par Charlotte Le Cornet et moi-même, à l'aide de la 'Newcastle-Ottawa Quality Assessment Scale'. Après exclusion des doublons, 72 articles ont été retenus : 65 portaient sur les expositions de l'adulte, sept sur les expositions prénatales ou parentales (deux articles en commun). Des associations avec un excès de risque de TGCT ont été retrouvées pour différents métiers (agriculteur, métier de la construction, pompier, policier, militaire, ouvrier des industries du papier, du métal et du plastique) et différentes expositions (champs électromagnétiques, polychlorobiphényls (PCB), et pesticides). Toutefois, les résultats pris dans leur ensemble sont discordants. A partir de la grille de lecture, on observe que les études montrant des associations positives avec les TGCT sont moins bien évaluées sur le plan de la qualité ($p=0.02$). Concernant les expositions prénatales, les pesticides semblent avoir été l'exposition la plus étudiée, mais les résultats semblent là aussi contradictoires.

Les limites méthodologiques des études peuvent en partie expliquer les contradictions dans les résultats observés. L'absence d'association évidente entre les expositions de l'adulte et les TGCT va dans les sens des hypothèses actuelles suggérant une origine prénatale de la maladie. Les futurs travaux devront se pencher non seulement sur les expositions prénatales aux pesticides, mais aussi sur la possible combinaison d'une exposition prénatale et d'une exposition survenant au cours de l'adolescence ou de la vie

adulte. La réalisation d'études collaboratives nationales ou internationales pourrait permettre de résoudre les problèmes de puissance liée à la rareté de la maladie. Des approches plus complètes pour évaluer efficacement les expositions de manière rétrospectives, de même que la recherche d'interactions gènes-environnements, seront nécessaires pour définir clairement le rôle des perturbateurs endocriniens.

SUMMARY - ENGLISH

Aim of the chapter: Although several studies have investigated potential risk factors for TGCT, no clear associations were found. Moreover, no literature review focused on prenatal exposures. The purpose of our review was therefore to summarize the current state of knowledge on occupational and environmental risk factors possibly associated with TGCT risk.

Systematic literature review: Using a systematic literature search of PubMed, we identified all articles published between 1990 and 2012 on this topic. All selected articles underwent a quality assessment by Charlotte Le Cornet and myself, using the 'Newcastle-Ottawa Quality Assessment Scale'. After exclusion of duplicate reports, 72 relevant articles were included; 65 assessed exposures during adulthood, 7 assessed parental exposures and 2 assessed both. TGCT risk has been linked to the occupations of agricultural workers, construction workers, firemen, policemen, military personnel, as well as to workers in the paper, plastic or metal industries. Exposures to electromagnetic fields, PCBs and pesticides have also been implicated. However, results were inconsistent and studies with positive associations tended to have lower quality rankings ($p=0.02$). Regarding prenatal exposures, pesticides represented the most frequent exposure examined, but their association with TGCT was not consistent across studies.

The studies' limitations may partly explain the inconsistencies observed. The lack of association with adulthood exposure is in line with current hypotheses supporting the prenatal origin of TGCT. Future researches should focus on prenatal or early life exposures, as well as combined effect of prenatal and later life exposures. National and international collaborative studies should allow more adequately powered

epidemiological studies. More sophisticated methods for assessing exposures retrospectively as well as evaluating gene–environment interactions will be necessary to establish clear conclusions.

II.2 (article #1)

Occupational and environmental exposures associated to testicular germ cell tumours: systematic review of prenatal and life-long exposures

Rémi Béranger [1, 2, 3], Charlotte Le Cornet [1, 2], Joachim Schüz [2], Béatrice Fervers [1].

(1) Unité Cancer et Environnement, Centre Léon Bérard, Lyon, France

(2) Section of Environment and Radiation, International Agency for Research on Cancer, Lyon, France

(3) Université Claude Bernard, Lyon, France

Article published in *PLoS One*

(2013 Oct 14;10(8): e77130)

OPEN ACCESS Freely available online

PLOS ONE

Occupational and Environmental Exposures Associated with Testicular Germ Cell Tumours: Systematic Review of Prenatal and Life-Long Exposures

Rémi Béranger[1,2,3*], Charlotte Le Cornet[1,2], Joachim Schüz[2], Béatrice Fervers[1]

1 Unité Cancer et Environnement, Centre Léon Bérard, Lyon, France, **2** Section of Environment and Radiation, International Agency for Research on Cancer, Lyon, France, **3** Université Claude, Bernard, Lyon, France

Abstract

Background: Testicular germ cell tumours (TGCT) are the most common cancers in men aged between 15 and 44 years and the incidence has increased steeply over the past 30 years. The rapid increase in the incidence, the spatial variation and the evolution of incidence in migrants suggest that environmental risk factors play a role in TGCT aetiology. The purpose of our review is to summarise the current state of knowledge on occupational and environmental factors thought to be associated with TGCT.

Methods: A systematic literature search of PubMed. All selected articles were quality appraised by two independent researchers using the 'Newcastle-Ottawa Quality Assessment Scale'.

Results: After exclusion of duplicate reports, 72 relevant articles were selected; 65 assessed exposure in adulthood, 7 assessed parental exposures and 2 assessed both. Associations with occupation was reported for agricultural workers, construction workers, firemen, policemen, military personnel, as well as workers in paper, plastic or metal industries. Electromagnetic fields, PCBs and pesticides were also suggested. However, results were inconsistent and studies showing positive associations tended to had lower quality ranking using the assessment scale (p=0.02).

Discussion: Current evidence does not allow concluding on existence of any clear association between TGCT and adulthood occupational or environmental exposure. The limitations of the studies may partly explain the inconsistencies observed. The lack of association with adulthood exposure is in line with current hypotheses supporting the prenatal origin of TGCT. Future research should focus on prenatal or early life exposure, as well as combined effect of prenatal and later life exposure. National and international collaborative studies should allow for more adequately powered epidemiological studies. More sophisticated methods for assessing exposure as well as evaluating gene–environment interactions will be necessary to establish clear conclusion.

Citation: Béranger R, Le Cornet C, Schüz J, Fervers B (2013) Occupational and Environmental Exposures Associated with Testicular Germ Cell Tumours: Systematic Review of Prenatal and Life-Long Exposures. PLoS ONE 8(10): e77130. doi:10.1371/journal.pone.0077130

Editor: Stefan Schlatt, University Hospital of Münster, Germany

Received August 6, 2013; **Accepted** September 6, 2013; **Published** October 14, 2013

Funding: RB recived a doctoral grant from the Rhône-Alpes region (grant n°12 008645 01; http://www.arc.rhonealpes.fr/). The authors received a grant from the French national cancer institute (grant n°2010-372; http://www.e-cancer.fr/en). The authors also received a grant from the 'Cancéropole CLARA' (no grant number; http://www.canceropole-clara.com). The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Competing interests: The authors have declared that no competing interests exist.

* E-mail : r.beranger26@gmail.com

Introduction

Testicular cancer is the most common cancer in men aged 15 - 44 years. Incidence rates have increased steeply in developed countries, with the highest incidence rates in Europe and in the USA [1]. Testicular germ cell tumours (TGCT) represent more than 90% of testicular cancer. Two main histological forms occur among young men: non-seminomas, which have an incidence that peaks at around 25 years old and seminomas that peak later, at around 35 years old. TGCTs in young adults should be distinguished from other rarer TGCTs histologies, which have different pathogenesis [2–4]: yolk sac tumours and immature teratomas occurring during childhood, and spermatocytic seminoma affecting mostly men over 50 years of age. The rapid increase and spatial disparities [1] of TGCT incidence as well as changes in incidence between first and second-generation immigrants [5–7] support a multifactorial origin of TGCT and in particular a role of environmental factors.

The possibility of an early life induction of TGCT is supported by the young age of cases, by the association with congenital abnormalities of the testis (cryptorchidism and hypospadias)

and results from numerous experimental studies suggesting that seminomas and non-seminomas could have a common precursor – the carcinoma in situ cell [4,8]. Testicular Dysgenesis Syndrome (TDS) has been proposed as the common origin for TGCT (except spermatocytic seminomas), cryptorchidism, hypospadias, and several types of decreased sperm quality. This syndrome may be caused by abnormal development of Sertoli and Leydig cells in the foetal testis resulting in delayed differentiation of germ cells and lower testosterone serum level during *in-utero* life [9]. Rare mutations, e.g. SRY mutations, can cause Testicular Dysgenesis Syndrome (TDS) but in most cases no mutations have been identified. It has been suggested that perinatal exposure to endocrine disruptors with estrogenic and anti-androgenic properties may play a role, particularly in individuals with genetic susceptibility to Testicular Dysgenesis Syndrome (TDS) [10]. Although this concept of TDS is currently controversial, the hypothesis of a prenatal (or early life) origin of TGCT is widely accepted [11,12].

So far, no animal models expressing TGCT type of the young adult have been found, although cases of spermatocytic seminomas have been reported. Our knowledge about TGCT risk factors is therefore based on epidemiological research [2]. Among the potential environmental factors, pesticides, which were first suggested to be a possible risk factor for TGCT in 1984 [13], appear to be one of the most studied. Available literature reviews [3,14–16] focused mainly on adulthood exposures and missed several articles. These generally provided few details on study methodologies and limits. Our systematic review aimed to critically analyse and evaluate available evidence from epidemiological studies to examine prenatal as well as life-long environmental and occupational exposures associated to TGCT.

Methods

Literature search

We followed the PRISMA statement for systematic reviews and meta-analysis for literature search, study selection, data extraction and synthesis (Checklist S1). A systematic review protocol was formalized with epidemiologist advisors (protocol not registered). Two independent investigators (RB and CLC) searched PubMed to identify relevant epidemiological studies on occupational and environmental risk factors for TGCT published between 1st January 1990 and 31st December 2012. Individual lifestyles factors (e.g. drugs, physical activity, tobacco, marijuana) were not included in this review.

The following search algorithm was used: (*"Testosterone/antagonists and inhibitors"[Mesh*] *OR "Endocrine Disruptors"[Mesh*] *OR "Pesticides"[Mesh*] *OR "Endocrine Disruptors"[TIAB*] *OR "Pesticides"[TIAB*] *OR "maternal exposure"[mesh*] *OR "environmental exposure"[mesh*] *OR "occupational diseases"[mesh*] *OR "occupations"[mesh*]) *AND* (*"Germinoma"[Mesh*] *OR "Testicular Neoplasms"[Mesh*] *OR "seminoma"[TIAB*] *OR "testicular dysgenesis syndrome"[tiab*] *OR "testicular cancer"[tiab*]).

Possibly relevant articles were selected through assessment of titles and abstract. Only original articles focusing on humans and written in English or French were kept in the review. Given the high survival rate of TGCT (more than 95% for localised tumours, 80% if metastatic), mortality studies lead to a selection of the population and were considered inappropriate for the purpose of our review [17]. To complete our literature search, we screened the reference lists of selected articles and related reviews.

For each publication, we abstracted the following information: first author's name; year of publication; journal; country of the studied population; study design; population size and characteristics (source, age structure, follow up, composition); approaches for exposure and outcome assessment; variable for stratification, groups matching or adjustment; and main results. When two or more publications reported data from the same study populations, we kept only the most detailed and/or the most recent publication. Publications with partially overlapping populations were retained when they provided complementary information.

Quality appraisal

Two researchers (RB and CLC) independently assessed the methodological quality of each study using the "Newcastle-Ottawa Quality Assessment Scale" (NOS). (http://www.ohri.ca/programs/clinical_epidemiology/oxford.asp). This scale has nine items in three parts: selection (four items), comparability (two items) and outcome (for cohort design, three items) or exposure (for case-control design, three items). The highest quality score a paper can obtain is '9'. In the event of disagreement, BF and JS provided input to obtain a consensus. Different publications issued from a same study might have diverging Newcastle-Ottawa Quality Assessment Scale (NOS) scores if the methodological aspects changed (e.g. adjustment factors, method for exposure assessment).

Statistical analyses

Statistical analyses were performed using SAS software package (version 9.3; SAS Institute Inc., Cary, NC, USA). The Newcastle-Ottawa Quality Assessment Scale (NOS) quality scores were compared using the Wilcoxon test.

Results

We identified 265 articles published between 1st January 1990 and 31st December 2012. We excluded 189 reviews, editorials and animal studies, 2 articles published in a language other than English or French, 23 publications that were out of scope, and 6 mortality studies. By analysing the reference lists of the 45 remaining articles and existing reviews on TGCT [3,14–16], we identified 35 additional publications responding to our inclusion criteria. Overall, 80 studies were selected and checked for potential overlap.

14 of the 80 selected publications reported data from the same study populations [20-33]. We kept the 6 most detailed or most recent of these for analysis [18–23]. Publications with partially overlapping populations were retained because they provided complementary information [24–26]: [27–29]; [30–32]; [33,34];; [35–38]. In total, we analyzed data from 72

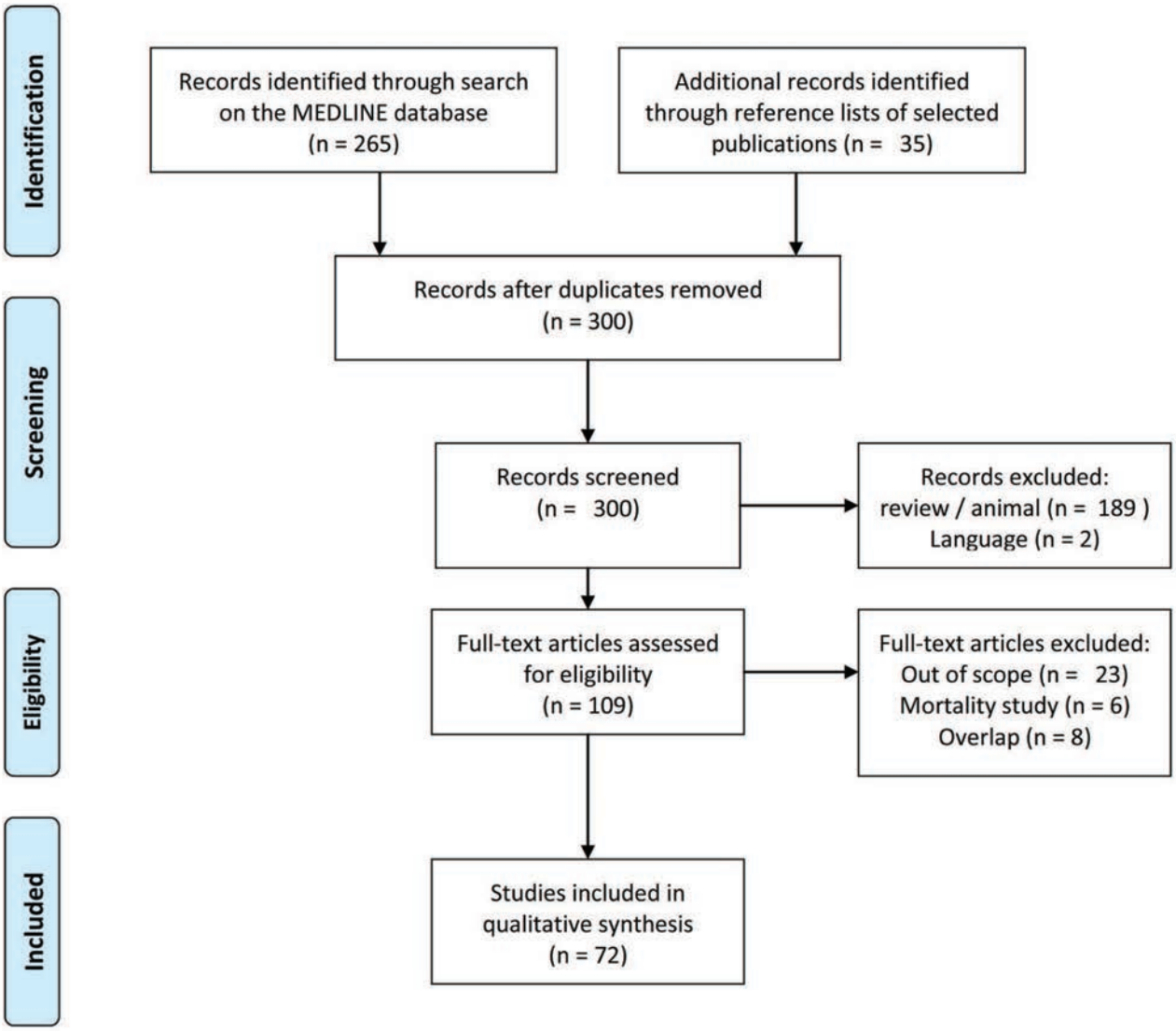


Figure 1. Flow diagram of identified articles published between 1990 and 2012.

doi: 10.1371/journal.pone.0077130.g001

publications for this review. Figure **1** represents the flow diagram of the articles selection.

The characteristics of the 72 selected articles and their NOS quality scores are summarized in Table **1** (two were ecological studies and thus not scored) [39,40]. The mean overall score was 6.3 out of 9 (2.6/4 for the selection part, 1.3/2 for comparability, 2.2/3 for outcome) with a high quality score (8 or 9) for eleven publications, an intermediate score (6 or 7) for 41 articles and a score of 5 or less for 18 publications. Studies published after 2000 were of higher quality than those published before 2000 ($p< 0.01$).

Of the 72 publications included in this review, 65 investigated exposure of the index subject (Table **S1**) and nine investigated parental exposure (Table **2**) (two focused both). No quality differences were found between these two types of studies ($p=0.36$). TGCT was the primary interest in 41 publications, while 31 investigated a broad range of cancers. Among the 23 studies investigating seminomas and non-seminomas separately, no risk factor appeared to be specific for one or other histologies. Studies reporting positive associations had lower NOS scores than those reporting negative results (6.02 versus 6.70, $p = 0.02$), mainly due to studies in which a positive association with TGCT was found for items other than the primary objective [20,41–45].

1. Index case exposure

Industrial exposure. Two of the six studies on workers in plastic-related industries [22,38,46–49], found an association with a risk for TGCT [22,49]. Statistically significantly increased risk of TGCT was found for Swedish plastic industry workers

Table 1. Description of selected studies.

Reference	Study design	Study population	Age limits	Exposure assessment	Diagnostic periods	Country	Quality assessment[a]			
							Sel. (4pt)	Com. (2pt)	Out. (3pt)	Total (9pt)
Alavanja 2005 [72]	Cohort[b]	57311 exposed / 27 cases	No details[c]	Questionnaire	1993 - 2002	USA	3	2	2	**7**
Andersson 2003 [54]	Cohort[b,e]	65637 exposed / 49 cases	31 - 84	Registry	1971 - 1990	Sweden	3	2	3	**8**
Andersson 2012 [57]	Cohort[b]	18113 workers / 26 cases	No details	Registry	1958 - 2001	Sweden	2	1	3	**6**
Band 2001 [55]	Cohort[b]	28278 exposed / 23 cases	No details	Registry	1950 - 1992	Canada	3	1	3	**7**
Bates 2001 [18]	Cohort[b]	3668 exposed / 11 cases	No details	Registry	1977 - 1995	New Zealand	3	1	2	**6**
Dement 2003 [59]	Cohort[b]	13354 exposed / 19 cases	No details	Registry	1979 - 2000	New Jersey	3	1	3	**7**
Dich 1996 [73]	Cohort[b]	20025 exposed / 18 cases	No details	Registry	1965 - 1991	Sweden	3	1	3	**7**
Finkelstein 1998 [65]	Cohort[b]	20601 exposed / 23 cases	No details	Registry	1964 - 1995	Ontario	1	1	2	**4**
Fleming 1999 [74]	Cohort[b]	30155 exposed / 23 cases	18 - 89	Registry	1981 - 1993	Florida	3	1	3	**7**
Floderus 1999 [30]	Cohort[b]	1596959 men / 607 cases	20 - 70	Registry	1971 - 1984	Sweden	2	0	3	**5**
Frost 2011 [75]	Cohort[b]	62960 exposed / 102 cases	No details	Registry	1987 - 2004	UK	2	1	2	**5**
Giles 1993 [62]	Cohort[b]	2865 exposed / 2 cases	15 / +[d]	Registry	1980 - 1989	Melbourne	3	1	3	**7**
Grayson 1996 [67]	Cohort[b]	227203 exposed / 59 cases	No details	Registry	1975 - 1989	USA	2	1	2	**5**
Guo 2004 [33]	Cohort[b,e]	667121 workers / 387 cases	25 / +[d]	Registry	1971 - 1995	Finland	3	2	3	**8**
Guo 2005 [34]	Cohort[b,e]	667121 workers / 387 cases	25 / +[d]	Registry	1971 - 1995	Finland	3	2	3	**8**
Gustavsson 2004 [97]	Cohort[b]	8750 exposed / 8 cases	No details	Registry	1989 - 1999	Sweden	3	1	2	**6**
Hansen 1996 [43]	Cohort[b]	10059 exposed / 4 cases	No details	Questionnaire	1968 - 1986	Denmark	1	1	3	**5**
Helmfrid 2012 [81]	Cohort[b]	641 cancers / 7 testis cancer	No details	Registry	1960 - 2003	Sweden	3	1	3	**7**
Hobbesland 1999 [53]	Cohort[b]	5918 exposed / 13 cases	No details	Registry	1953 - 1991	Norway	1	1	3	**5**
Kelleher 1998 [76]	Cohort[b]	About 150000 men / 47 cases	16 - 65	Registry	1980 - 1990	Ireland	3	1	1	**5**
Kristensen 1996 [21]	Cohort[b]	166291 exposed / 158 cases	0 - 39	Registry	1965 - 1991	Norway	3	2	2	**7**
Kristensen 2000 [98]	Cohort[b]	47285 men and 36787 son exposed / 70 and 63 cases	0 - 70	Registry	1967 - 1995	Norway	2	2	2	**6**
Langard 2000 [47]	Cohort[b]	428 exposed / 1 cases	No details	Registry	1953 - 1993	Norway	3	1	3	**7**
Ma 2006 [63]	Cohort[b]	34796 exposed / 54 cases	18 / +[d]	Registry	1981 - 1999	Florida	2	1	3	**6**
Milanov 1999 [68]	Cohort	52963 person-year / 6 cases	No details	Registry	1964 - 1994	Bulgaria	1	1	3	**5**
Rix 1998 [56]	Cohort[b]	11130 men / 29 cases	No details	Registry	1943 - 1996	Denmark	3	1	3	**7**
Rodval 2003 [85]	Cohort[b]	About 14000 exposed / 2 cases	0 - 36	Registry	1958 - 1994	Sweden	3	1	2	**6**
Pollan 2001 [31]	Cohort[b]	1779646 men / 1189 cases	24 - 79	Registry	1970 - 1989	Sweden	3	2	3	**8**

Table 1 (continued).

Reference	Study design	Study population	Age limits	Exposure assessment	Diagnostic periods	Country	Quality assessment[a]			
							Sel. (4pt)	Com. (2pt)	Out. (3pt)	Total (9pt)
Sigurdson 2003 [83]	Cohort[b,f]	20781 exposed / 16 cases	No details	Questionnaire	1983 - 1998	USA	2	2	3	**7**
Sonneveld 1999 [23]	Cohort[b]	7473676 men / 2591 cases	No limits	Registry	1989 - 1995	Netherland	3	1	2	**6**
Sulem 2003 [99]	Cohort[b]	3874 exposed / 0 cases	18 / +[d]	Registry	1968 - 1998	Iceland	2	1	3	**6**
Tynes 1992 [60]	Cohort[b]	37945 exposed / 41 cases	20 / +[d]	Registry	1961 - 1985	Norway	3	1	3	**7**
Yamane 2006 [71]	Cohort[b,f]	From 281604 to 489590 exposed / 354 cases	18 - 51	Registry	1989 - 2002	USA	2	2	3	**7**
Zandjani 1994 [77]	Cohort[b]	1756 exposed / 7 cases	No details	Registry	1953 - 1992	Norway	3	1	3	**7**
Bates 2007 [61]	Case-control[b,e,f]	70 cases / 804107 controls	21 - 80	Registry	1988 - 2003	California	2	2	2	**6**
Baumgardt-Elms 2002 [24]	Case-control	269 cases / 797 controls	15 - 69	Interview	1995 - 1997	Germany	3	2	2	**7**
Baumgardt-Elms 2005 [25]	Case-control	145 cases / 196 controls	15 - 69	GIS	1995 - 1997	Germany	3	1	3	**7**
Biggs 2008 [78]	Case-control	272 cases / 726 controls	18 - 44	Biol. sample	1999 - 2008	Washington	4	1	2	**7**
Bullman 1994 [41]	Case-control[b]	97 cases / 311 controls	28 / +[d]	Registry	1982 - 1991	USA	1	0	2	**3**
Chia 2010 [27]	Case-control[f]	577 cases / 707 controls	18 - 45	Biol. sample	1988 - 2003	USA	4	2	3	**9**
Foley 1995 [66]	Case-control[b]	148 cases	17 - 49	Registry	1984 - 1989	UK	3	1	2	**6**
Giannandrea 2011 [42]	Case-control[e]	50 cases / 48 controls	18 - 45	Interview + biol. sample	2006 - 2008	Italia	2	2	2	**6**
Hansen 1999 [46]	Case-control[b]	3745 cases / 7490 controls	16 - 75	Registry	1970 - 1989	Denmark	3	2	2	**7**
Hardell 2006 [19]	Case-control	61 cases / 58 controls	18 - 45	Biol. Sample	1997 - 2000	Sweden	3	1	2	**6**
Hardell 2004 [49]	Case-control	791 paired cases and controls	20 - 75	Questionnaire	1993 - 1997	Sweden	3	1	2	**6**
Hayes 1990 [35]	Case-control	266 cases / 271 controls	18 - 42	Interview	1976 - 1981	Washington	2	2	2	**6**
Kardaun 1991 [36]	Case-control	308 cases / 288 controls, 225 and 212 mothers, respectively	18 - 42	Interview	1976 - 1981	Washington	2	1	2	**5**
Knoke 1998 [20]	Case-control[b,f]	134 cases / 371 controls	17 - 65	Registry	1990 - 1996	USA	2	2	1	**5**
Knight 1996 [51]	Case-control[e]	495 cases / 974 controls	16 - 59	Questionnaire	1987 - 1989	Ontario	3	2	1	**6**
Knight 1997 [87]	Case-control[e]	495 cases / 974 controls, 343 and 524 mothers, respectively	16 - 59	Questionnaire	1987 - 1989	Ontario	3	1	1	**5**
Marshall 1990 [82]	Case-control	18 cases / 259 controls	20 - 54	Registry	1974 - 1986	New York	2	1	1	**4**
McGlynn 2009 [29]	Case-control[f]	736 cases / 913 controls	18 - 45	Biol. Sample	1988 - 2003	USA	4	2	3	**9**
McGlynn 2008 [28]	Case-control[f]	739 cases / 915 controls	18 - 45	Biol. Sample	1988 - 2003	USA	4	2	3	**9**
Moller 1997 [80]	Case-control[f,g]	296 cases / 287 controls	16 - 42	Questionnaire	1986 - 1988	Denmark	3	2	1	**6**
Nori 2006 [44]	Case-control[e,f,g]	103 cases / 215 controls ; 63 and 123 mothers, respectively	18 / +[d]	Interview	1996 - 2003	Italia	2	2	1	**5**
Ohlson 2000 [22]	Case-control	148 cases / 314 controls	30 - 75	Questionnaire	1989 - 1992	Sweden	3	1	2	**6**
Rhomberg 1995 [52]	Case-control	165 cases / 187 controls	18 / +[d]	Interview	1971 - 1978	Germany	3	0	0	**3**
Ryder 1997 [69]	Case-control[b]	110 cases / 440 controls	15 - 59	Registry	1976 - 1994	UK	4	1	3	**8**
Stang 2003 [26]	Case-control[g]	269 cases / 797 controls	15 - 69	Interview	1995 - 1997	Germany	3	2	1	**6**
Stenlund 1997 [32]	Case-control[b]	134 cases / 1121 controls	25 -70	Registry	1985 - 1987	Sweden	3	2	2	**7**
Swerdlow 1991 [45]	Case-control	259 cases / 489 controls	10 / +[d]	Interview	1977 - 1981	UK	1	1	1	**3**
Tarone 1991 [37]	Case-control[g]	156 cases / 130 controls	18 - 42	Interview	1976 - 1981	Washington	2	2	2	**6**

Table 1 (continued).

Reference	Study design	Study population	Age limits	Exposure assessment	Diagnostic periods	Country	Quality assessment[a]			
							Sel. (4pt)	Com. (2pt)	Out. (3pt)	Total (9pt)
Van der Eeden 1991 [38]	Case-control[f,g]	390 cases / 729 controls	20 - 69	Interview	1977 - 1984	Washington	4	2	2	**8**
Walchaert 2007 [48]	Case-control[g]	229 cases / 800 controls	20 - 45	Questionnaire	2002 - 2005	France	2	1	1	**4**
Yamane 2003 [70]	Case-control[b,f]	74 cases / 296 controls	23 - 55	Registry	1989 - 1999	USA	2	2	3	**7**
Zhang 1995 [84]	Case-control[e,g]	250 cases and control	15 / +[d]	Interview	1977 - 1980	New York	3	2	1	**6**
Behrens 2012 [50]	Nested case-control	169 cases / 988 controls	No details	Interview	1989 - 2006	Germany	4	2	2	**8**
Cohn 2010 [86]	Nested case-control[f]	15 cases / 45 controls	17 - 37	Biol. sample	1957 - 2000	California	3	2	3	**8**
Purdue 2009 [79]	Nested case-control	49 cases / 51 controls	No details	Biol. Sample	1972 - 1999	Norway	3	1	3	**7**
Koifman 2002 [39]	Ecological study	No details	0 - 49	N/A	1999 - 2000	Brazil	N/A	N/A	N/A	
Mills 1998 [40]	Ecological study[f]	No details	No details	N/A	1988 - 1992	California	N/A	N/A	N/A	
Davis 1993 [100]	Cluster	6 case / 340 controls	27 - 47	Interview	1979 - 1991	Washington	1	0	3	**4**

Abbreviations: sel. = Selection; Com. = comparability; out. = outcome. Biol. Sample = biological sample. N/A = not applicable; GIS = Geographic Information System.

"Questionnaire" means self-administered questionnaire, in contrast to "Interview". All studies were stratified or adjusted on age but four [35,41,52,100].

a *The quality score was determined by using the Newcastle-Ottawa quality assessment scale*;

b Registry-based studies.

c *Information not provided by authors*

d *No upper age limit.*

e Adjustment on socioeconomic status

f *Adjustment on ethnicity or study focusing only on Caucasian*

g Adjustment on (or exclusion of) cryptorchidism

doi: 10.1371/journal.pone.0077130.t001

exposed to polyvinyl chloride (PVC) but this association was based on analyses in a small sub-group [22]. Using a larger population, the same team observed an association between PVC and TGCT, but only in the group with the lowest level of exposure and for less than 8 years of cumulative exposure [49]. Stratification for the time between first exposure and diagnosis did not show any specific trend. The authors concluded that the association was probably due to random cluster [49]. In addition, no association with TGCT was seen for other plastic components, such as styrene, urethane or acrylate [22], as well as for chemical industry related tasks [38,48].

Five of the eight identified studies found significant associations with occupations in metal industries [31,38,50,52,53]. However, metalworking occupations and associated exposures investigated varied importantly across studies (e.g. metal trimming, metal annealer, welding) [31,38,43,48,50–53], making comparisons difficult. Occupation as a precision metal worker was found to be associated with TGCT while occupation as metal machine operators or metal making workers were not [38]. An increased risk of TGCT was found for furnace workers in a ferrosilicon plant, but no association with duration of work was reported [53]. In a nationwide study in Sweden a significantly higher risk of seminomas was reported for metal annealer and/or temperer workers, whereas no association was found for precision toolmakers, metal casters and moulders or other metal processing workers [31]. The two remaining studies showed an excess of seminomas among metalworkers [52] and of non-seminomas in a sub-group of automobile workers involved in metal-cutting tasks [50]. However the first one should be interpreted cautiously because of methodological limitations (questionnaires differed between cases and controls, no consideration of age, imprecise exposure assessment) [52]. A French case-control study reported an increased risk of TGCT for workers involved in welding and TGCT risk but this was no longer significant after adjustment for potentially confounding factors [48]. In Denmark, an increased risk of TGCT in stainless steel grinding workers was observed (based on 4 cases), but not for other metalworkers (including welders) [43]. The authors concluded that the association might be due to confounding by socio-economic status (SES).

Seven articles investigated the association between working in the paper industry and TGCT [34,38,45,54–57] with divergent results. A Swedish registry-based cohort study reported a positive association for seminomas depending on

Table 2. Parental occupational and environmental exposures related to testicular germ cell cancer in offspring.

Exposure categories	*Ref.*	*Exposure*	*Time of cases exposure*	*All testicular cancer*	*Seminoma*	Non-seminoma
Occupational exposures						
Agricultural workers						
Agriculture, forestry	[36]	Paternal	Childhood	OR = 0.4 [0.1-0.9]	-	-
Agriculture, forestry	[36]	Paternal	Prenatal	OR = 0.9 [0.4-1.8]	OR = 0.7 [0.1-2.7]	-
Employed in agriculture	[80]	Maternal	Prenatal	OR = 1.23 [0.56-2.69]	OR = 1.34 [0.50-3.57]	OR = 1.32 [0.51-3.40]
Employed in agriculture with animals	[80]	Paternal	Prenatal	OR = 0.64 [0.42-0.99]	OR = 0.61 [0.34-1.08]	OR = 0.68 [0.40-1.16]
Engaged in agriculture activity	[21]	Parental	-	SIR = 124 [1.1-152]	-	-
Engaged in agriculture activity	[21]	Parental	-	OR = 2.44 [1.66-3.56]	OR = 1.70 [0.81-3.57]	OR = 4.21 [2.13-8.32]
Application of ≥100kg nitrogen /hectare	[21]	Parental	-	RR = 1.84 [1.22-2.76]	-	-
Farm worker	[42]	Parental	Prenatal	p = 0.35		
Pesticide applicators	[85]	Parental	-	OR = 1.19 [0.13-4.28]	-	-
-	[42]	Parental	Prenatal	p = 0.63		
Metal worker						
Metalworkers	[87]	Paternal	Year before conception	OR = 3.28 [1.03 - 10.52]	-	-
Metal products	[87]	Paternal	Year before conception	OR = 5.77 [1.53 - 21.77]	-	-
Wood workers						
Wood processors	[87]	Paternal	Year before conception	OR = 10.46 [1.20 - 91.14]	-	-
Health related	[36]	Paternal	Childhood	OR = 3.9 [0.4-190.7]	OR = 5.1 [0.1-405.8]	-
-	[36]	Paternal	Prenatal	OR = 1.4 [0.2-17.3]	OR = 5.2 [0.4-73.6]	-
-	[36]	Maternal	Prenatal	OR = 1.4 [0.4-5.0]	OR = 4.6 [1.1-19.1]	-
-	[87]	Maternal	Before conception	OR = 0.54 [0.26 - 1.13]	-	-
Food producers						
Food and beverage services industry	[87]	Paternal	Year before conception	OR = 4.36 [1.50 - 12.63]	-	-
Food products	[87]	Paternal	Year before conception	OR = 2.79 [1.34 - 5.79]	-	-
Chemical exposures						
Organochlorines						
HCB	[19]	Maternal[a]	At son's diagnostic	OR = 4.4 [1.7–12]	-	-
p,p'- DDT	[86]	Maternal[a]	1-3 days after delivery	OR = 0.70 [0.26-1.64]	-	
o,p-DDT	[86]	Maternal[a]	1-3 days after delivery	OR = 0.77 [0.37-1.33]	-	-
p,p'- DDE	[86]	Maternal[a]	1-3days after delivery	OR = 0.19 [0.04-0.62]	-	
	[19]	Maternal[a]	At son's diagnostic	OR = 1.3 [0.5–3.0]	-	-
Total chlordanes	[19]	Maternal[a]	At son's diagnostic	OR = 1.9 [0.7–5.0]	-	-
Ratio p,p'-DDT/ p,p'-DDE	[86]	Maternal[a]	1-3days after delivery	OR = 3.56 [1.34-11.88]	-	
PCB						
Sum of PCBs	[19]	Maternal[a]	At son's diagnostic	OR = 3.8 [1.4–10]	OR = 3.1 [0.7–14]	OR = 4.3 [1.3–14]
Estrogenic PCBs	[19]	Maternal[a]	At son's diagnostic	OR = 2.4 [0.95–6.0]	OR = 2.3 [0.6–8.9]	OR = 2.4 [0.8–6.8]
Enzyme-inducing PCBs	[19]	Maternal[a]	At son's diagnostic	OR = 2.6 [1.03–6.5]	OR = 1.4 [0.4–5.3]	OR = 3.3 [1.1–9.7]
Toxic equivalents (TEQ)	[19]	Maternal[a]	At son's diagnostic	OR = 3.3 [1.3–8.4]	OR = 3.5 [0.8–15]	OR = 3.3 [1.1–9.8]
Sum of PBDE	[19]	Maternal[a]	At son's diagnostic	OR = 2.5 [1.02–6.0]	OR = 1.8 [0.5–6.5]	OR = 2.9 [1.04–8.2]
Endocrine-disrupting chemicals	[44]	Maternal	Prenatal	OR = 0.97 [0.23-4.07]	OR = 0.99 [0.16-6.07]	OR = 1.13 [0.19-6.86]
-	[44]	Paternal	Prenatal	OR = 1.33 [0.65-2.70]	OR = 1.24 [0.51-3.01]	OR = 1.42 [0.55-3.67]
Others						
Residency urban/rural	[44]	Parental	During fetal life	OR = 1.35 [0.49-3.71]	OR = 1.54 [0.44-5.35]	OR = 1.29 [0.34-4.94]
Social class (professionals vs. manual workers)	[45]	Paternal	At birth	OR = 1.48 [0.69-3.16]	-	-

Abbreviations: HCB = Hexachlorobenzene, DDT = dichlorodiphenyltrichloroethane; DDE = Dichlorodiphenyldichloroethylene; PCB = polychlorinated biphenyl; PBDE = *Polybrominated diphenylethers*.

a Exposure assessment by biological samples, in contrast to questionnaire/registry-based exposure assessment.

doi: 10.1371/journal.pone.0077130.t002

the duration of employment [54]. This study considered only workers over 30 years of age to guarantees a minimum of cumulative exposure, thus, some TGCT cases may have been missed. Another Swedish registry-based cohort study showed a positive association for sulphate pulping workers, as well as sulphate mills workers but only for less than 10 years of exposure and latency [57]. Conversely, a Danish nationwide registry-based cohort study [56] and a Canadian cohort study did not find any association with occupation in the paper industry. Yet, the latter was based on 23 cases only [55]. The

three remaining studies that investigated various occupational exposures did not find any association for paper industry workers with TGCT [34,38,45].

White-collar workers, professionals and higher social-economic-status (SES). An excess risk for TGCT was reported for several white-collar or professional occupations [31,35,38]. Some authors suggested that this might be due to SES rather than occupational exposure [35,38]. Guo et al. reported an excess risk for university teachers, electrical engineers, system analysts and programmers [34] but these associations disappeared after adjustment for SES. Swerdlow et al. found an association for higher SES but not for white-collar occupations [45]. The results from Knight et al. suggested an association with SES mainly among seminoma cases [51], whereas a Danish study reported no association for SES but found an association for ethnicity [58].

Construction and related occupations. Eight studies investigated TGCT risk in construction and related occupations [22,31,35,38,45,48,59,60]. Two focused specifically on construction workers, with inconsistent results [31,35]. Electrical workers had an elevated risk in one study [38] out of four [22,38,45,60]. No association was found for painters [38,45,48]. One study on carpenters in the US reported an increased risk, but only for workers employed from 0 to 10 years, considering 15 years of latency [59]. However, comparison with the general New Jersey population might have led to an overestimation of the risk, since 97.3% of carpenters were Caucasian. In addition, no association was found for wood workers [45], lumber-jacks [38] and workers exposed to wood dust.

Firemen, policeman and military workers. Five studies investigated TGCT risk in firemen [18,26,61–63] with divergent results. Excess risk was reported for fire-fighters in three studies: two cohort studies from New Zealand [18] and Florida [63] and one case-control study from California (for 1988-1995 but not for 1996-2003) [61]. Two additional studies conducted in Australia (cohort) [62] and Germany (case-control) [26] did not found any association. However, the sample sizes in these two studies were small. All five studies were registry-based and did not consider potential additional exposures.

Four studies investigated the risk in policemen [31,33,64,65] with divergent results. Davis et al. found a TGCT cluster in Ontario and suggested this was due to electromagnetic field exposure resulting from the use of radar devices [64]. Finkelstein et al. also found a positive association, but used a 90% interval confidence[65]. A Swedish population-based study found a significant association with seminomas in policemen compared to the general population [41]. This association disappeared when compared with other occupations in the same sector (services and military work) or when policemen potentially exposed for longer periods were considered [31]. A fourth study failed to show any association [33].

Ten studies investigated TGCT risk in military and related occupations [20,31,37,41,45,66–70] with inconsistent results. Of the five studies investigating general military workers [20,31,45,66,69], only one reported a weak association with seminomas [31]. Five studies reported an elevated risk of TGCT for air force personnel [20,66,67,69,70] while four others did not [37,41,68] or suggested a protective effect [71]. Similar inconsistent associations were observed in studies on navy personnel [20,37,41]. Studies on American Marine Corps personnel did not report an association with TGCT [20,37,41,69]. Overall, risk excess in these studies was observed for personnel involved in specific tasks (electric, mechanic or maintenance) [20,66,69]. Non-seminoma tumours were found associated with having been in service in Vietnam [37], but not with Agent Orange exposure during the Vietnam War [41].

Farmers, agricultural workers and occupational exposures to pesticides. Overall, 15 studies investigated TGCT risk among agricultural workers, pesticides applicators or in occupations associated with pesticides exposures [31,34,35,38,42,44,45,48,51,72–77]. On the six studies assessing occupational exposures to pesticides in general, no significant associations were reported [34,35,42,44,45,51], except for one study showing a protective effect for seminoma tumours [51]. However, this study was based on self-reported exposures, small subgroups, and numerous statistical tests were performed without correction for multiple testing. On the six studies investigating agricultural occupations [31,34,35,38,45,76], only one found an increased risk, but mainly due to a small subgroup of fish farmers [76]. On the four registry-based studies focusing on licensed pesticide applicators, an increased TGCT risk was reported for a Floridian [74] and a UK study [75], but not for a Swedish study [73] or for the US Agricultural Health Study [72]. These studies may be affected by misclassification bias since unregistered agricultural workers, under the supervision of licensed farmers, may have applied the pesticides. A Norwegian study identified a slight excess risk for workers in a fertilizer production plant, but no association with exposure to a particular product [77]. Since the plant was located in a rural area, it was suggested that this was the potential impact of life-long environmental exposure to other factors, however not investigated by this study. No associations were reported for workers in the pesticide industry [48].

Magnetic and electric field exposure. Five studies investigated magnetic and electric field exposures [24,30,32,35,48]. Among these, two Swedish population-based studies reported a positive association between magnetic fields and TGCT risk (1971-1984 [30] and 1985-1987 [32]) using the same job-exposure matrix (JEM). The first reported a higher risk for the medium level of exposure and younger workers [30], the second found a dose-response effect for non-seminomas and workers under 40 years of age, but not for the others [32]. A US case-control study found a positive association for self-reported occupational exposure to microwaves and other radiowaves, but this association disappeared when the exposure was estimated independently using job titles [35]. No association was reported for radar equipment use [24,35,48] nor for working near visual display units or ‘complex electronic environment’ [24]. Radar exposures were also investigated in studies on policemen (see above), but no clear association was reported.

Environmental exposures to organochlorines and pesticides. Eight studies investigated environmental exposure to organochlorines using blood samples [19,27–29,42,44,78,79] or questionnaires [42,44]. The study by McGlynn et al. suggested that p,p'-DDE, oxychlordane, cis-nonachlor, trans-nonachlor and total chlordane serum levels are associated with TGCT risk, especially with seminomas [28]. Other studies did not report any association with trans-nonachlor [78,79], total chlordanes [19], and p,p'-DDE serum levels [19,42,78,79]. Two polymorphisms of the CYP1A1 gene (rs7495708 and rs1456432) were suspected to be associated with an increase of TGCT risk in men having elevated total chlordane serum levels [27]. Overall, no association was observed with serum levels of hexachlorohexane [28,78], hexachlorobenzene (HCB) [19,78], pp'DDT, oxychlordane [28,78,79], op'DDT [78,79] and mirex [28,79]. McGlynn et al. reported a protective effect for PCBs serum levels, either individually or grouped [29], while others studies showed mixed effects (increased or decreased risk) [79], or no association [19]. However, some authors suggest there is uncertainty on the conclusions to be drawn from observed association between organochlorine levels measured in adulthood and past exposures occurred during early (or prenatal) life since important physiological variations occur over life, especially at puberty [78]. Moreover, exposure may have occurred after the in utero or infancy period and genetic polymorphisms in metabolism may also have an impact on the serum concentrations [27].

One study suggested an increased risk for self-reported domestic insecticides use, but blood analyses failed to confirm this association [42]. Using a JEM, an Italian study reported an excess TGCT risk associated with hobbies involving exposure to endocrine disrupting chemicals during adolescence, but not for occupations involving the same exposure [44].

It has been suggested that living in rural areas could be a surrogate for environmental exposure to pesticides [21–23,44,48,80]. One study found an increased risk of TGCT among men who reported living in a rural area (defined as living <1 km from a farm) during adolescence [44], while others showed a protective effect [80] or no association [21,22]. Inconsistent associations with TGCT risk have also been reported for rural area residency during adulthood [22,23,44,48]. Additionally, a Swedish registry-based cohort suggested a non-significant increased risk for men living in an area contaminated by PCBs and metals [81].

Risk associated with miscellaneous occupations and exposures. Leather workers exposed to dimethylformamide (DMF) have been suspected to be at risk for TGCT based on the report of a small cluster in a New York plant [82]. Association was confirmed for non-seminomas in a second study [51], but not in two larger studies assessing various occupational exposures [34,45]. Occupations related to food processing were reported to be associated with an increased risk for TGCT in two studies investigating a wide range of occupations [38,51]. The authors suggested a role of cleaning agents, disinfectant and insecticides. Exposure to polycyclic aromatic hydrocarbons and hydrocarbons such as diesel and gasoline was not reported to be associated with a risk for TGCT [33–35,38]. Likewise exposures to radioactive material and nuclear activity were also not reported to be associated with risk for TGCT [35,48,69,83]. A US case-control study reported that worker exposed to 'extreme temperatures' (lower than 60°F (15°C) or higher than 80°F (27°C)) have an increased risk of TGCT, however exposure was self-reported and the response rate of cases was low (<40%) [84].

2. Parental exposure

Among the 9 studies investigating occupational and environmental parental exposure, agriculture-related parental exposure has been the most studied (5 studies). No excess risk for TGCT was reported among the sons of farmers or pesticide applicators [36,42,80,85], except in one study in Norway that reported an increased risk for sons of agricultural workers using nitrate fertiliser, especially with a high nitrate/phosphate ratio [21]. In this study, the adjusted risk was higher for exposed men born between April and June or October and December. Since the high nitrate/phosphate ratio is related to intensive farming, association with TGCT might be linked to specific related exposure patterns, including pesticides use, type of farming or farming practices. In a Danish study, Moller et al. suggested an increased risk associated with childhood residence in a high-nitrate area, but only for those who did not grow up on a farm [80]. Moreover, residence in a farm during childhood was associated with a protective effect. The authors concluded that nitrates are unlikely to be responsible for the observed TGCT risk excess. Another study on pesticide applicators' children in Sweden did not report an association, however only two cases of TGCT were reported and the follow up was short (the median age at end of the follow-up was 20 to 24 years) [85]. In addition, about 25% of the sons were already born when their fathers obtained their pesticide applicator's license.

A positive association was found for hexachlorobenzene, PCBs, PBDE and chlordanes in maternal serum and the risk of TGCC among the women's sons [19]. However, maternal blood samples were collected at time of sons' TGCT diagnosis and selection bias may have occurred since cases were chosen by their physicians. In a small US nested case-control study (15 TGCT cases and 45 controls), in which the maternal blood samples have been collected during pregnancy and a few days after delivery, no association was reported between TGCT risk in sons and the mother's DDT or DDE serum levels [86]. The authors suggested that, compared with controls, the mothers of cases may be slower to eliminate DDT.

Other self-reported occupations in parents were reported to be associated with TGCT (e.g. healthcare professions, wood and metal-workers, working in food production) but these studies performed numerous statistical tests, had small subgroups and were exposed to possible selection bias [36,87]. Overall, in the few studies focussing on TGCT in the offspring, the methods used to assess parental exposure had limitations.

Discussion

To our knowledge, this is the most comprehensive review of studies published in the past two decades on environmental and occupational exposures possibly associated with TGCT, for prenatal childhood and adulthood periods. In contrast to previous reviews on TGCT [14–16,29], we considered that current evidence is inconsistent and does not allow to conclude on existence of any clear association between TGCT and adulthood occupational or environmental exposure. This is in line with current hypotheses suggesting that TGCT may originate from in utero or early life exposure [4,9]. However, very few studies investigated the impact of parental occupational or environmental exposure, and results were inconsistent.

For the first time, this review include systematic quality appraisal for all studies. Studies reporting a positive association with TGCT had significantly lower quality scores than studies showing no association. Also, some of the inconsistent results may be explained by methodological limitations or study design, as developed below.

Inconsistent associations

The low incidence of TGCT constitutes a barrier to conducting adequately powered epidemiological studies. None of the publications provided information on the minimum detectable risk based on the study population size. Lack of power could partly explain the inconsistent results reported. Furthermore, some positive associations may be due to chance, when multiple testing is performed without correction (e.g. more than 300 tests were performed by Pollan et al.) [31]. Some very high Odds Ratios have been identified (e.g. OR = 14 [IC95% 2.8-75] [19]). These results might be related to outliers or interaction problems and should be interpreted with caution. Also, several publications derived from investigations within cancer registries. Since incidence excess in cancer registry leads generally to investigations, publication bias is likely because TGCT incidence would not be explored systematically in registries where incidence is normal.

Potential confounders

Large variations in TGCT risk have been reported for different ethnicities [89], with the highest risk being in Caucasian men. This factor was rarely assessed in the identified studies and Caucasian men could be overrepresented in some occupations such as policemen, firemen, military employees, farmers or jobs related to higher SES and might explain some associations observed. SES was generally estimated using indirect means such as income or education level and shows inconstant association with TGCT [35,45,88]. A large Danish population-based study in men over 30 years old reported no significant association for income, education level or 'Charlson Comorbidity Index', but only for ethnic origin [58]. Otherwise, cryptorchidism is an established risk factor for TGCT and several studies adjusted for this. However, according to the TDS hypothesis [9], cryptorchidism and TGCT may have a common underlying early developmental cause. In this case, the two factors are collinear and will have no impact on their mutual risk. Adjustment may therefore have weakened any potential association. Furthermore, several polymorphisms have been recently associated with the risk of TGCT [10,27], but rarely considered in the published studies.

Exposure assessment

Heterogeneity of occupations and definitions of exposures may further explain inconsistencies across studies, and did not allow us to perform a pooled analysis. Moreover, while for some occupations the job title can be used as surrogate exposure variable (e.g., welders and welding fumes), the reliability of exposure assessment is limited for others (e.g., farmers and pesticides exposure). Misclassification of pesticide exposures could have resulted in weaker association since some farmers should not have been classified as exposed, whereas other occupations associated with pesticides exposure (e.g., carpenters, sawmill workers) in the general population were classified as not being exposed [90]. In addition, very few studies assessed the association with domestic exposure to pesticides (i.e. gardening, indoor use of insecticide spray) and those that did were mainly based on self-reported exposure. The whole population is exposed to ubiquitous pollutant, at least a minimum, making it difficult to identify control groups to assess related adverse effects in absence of precise exposure assessment.

Self-reported exposures can provide detailed information, but potential recall bias may lead to over-estimation in comparison to JEM or independent assessment [91]. The evolution of industrial practices and occupational exposure could further explain inconsistency between previous studies and more recent ones.

Plausibility of adult exposure for the index subject

TGCT occurs mainly in young adults, a population with lower cumulative occupational exposure and shorter time-lag between occupational exposure and cancer diagnosis. Thus, occupational exposure of the index subject may not be relevant. While uncertainties remain concerning the exact window of exposure associated with occurrence of TGCT, events during the 'testicle programming period' has been proposed to originate in cryptochidism and hypospadias in rats [92]. This is an interesting concept since these diseases are thought to have common underlying causes with TGCT in men. Extrapolating to humans, this period would correspond to the 8^{th} to 14^{th} week of pregnancy, but differences in the reproductive mechanisms of rodents and human may limit extrapolations [93,94].

In contrast, environmental or occupational exposure during adulthood might be associated with spermatocytic seminomas that occurring mainly after 50 years: the cumulative exposure is higher, particularly for occupational exposure, the latency periods are long enough, and the related precursor cells appeared only after puberty and during adulthood [2]. The spermatocytic seminomas subtype accounts for up to 4% of all TGCT [2] but this proportion can increase when the age limit of the sample at inclusion is higher. Since spermatocytic seminomas are generally grouped with classic seminomas, it is

possible that this explains some associations between adult exposure and TGCT that are reported. To avoid potential bias, spermatocytic seminomas should be considered separately from other TGCT.

Limit of the review

The NOS quality assessment scale has been criticized for potential inter-operator variability [95,96]. In response to these limitations, independent evaluation by two investigators combined with arbitrage has been performed to increase reliability of scoring.

While the majority of publications in our review were identified through PubMed, additional studies were identified through the reference lists of relevant articles and recent reviews. The latter mainly focused on multiple cancer sites where TGCT was not the primary cancer of interest, and thus, not apparent in keywords of abstracts. However, we can be confident that the combination of these two methods has enabled us to identify all the pertinent studies on environmental or occupational risk factors for TGCT and the vast majority of studies exploring a wider range of cancers including TGCT.

Recommendations

The study limitations discussed above makes it difficult to detect or to interpret associations. Future studies on TGCT should consider intra-uterine and parental exposure, since it is plausible that exposure during early development leads to TGCT and this has been insufficiently explored. A combined effect of prenatal and postnatal, adolescent or adulthood exposure has also been suggested [16], but has not been explored yet. Moreover, we recommend that domestic, environmental and occupational exposure should be assessed in future studies to minimise misclassification bias, as well as genetic and molecular biology techniques for evaluating gene–environment interactions. Quantitative exposure assessment should be improved when considering ubiquitous pollutants (e.g.: use of biomarkers or geographic information systems). Finally, to overcome problems linked with the lack of statistical power, we recommend the use of more standardized approaches in future studies to allow meta-analyses or pooled studies. This could be achieved by the creation of consortia that can give guidance on the design and analyses of next generation studies.

A national case-control is currently conducted in France (TESTIS project) to explore the impact of life-time pesticide exposure on TGCT risk using combined methods including job exposure assessment by experts, geographical information system technology and polymorphism analyses.

Conclusions

Despite of the numerous factors investigated in many studies, the reasons for the rapid increase of TGCT incidence remain unclear. Occupational exposures during adulthood are unlikely to be involved in TGCT aetiology because of the young age of patients. The lack of convincing association with adulthood exposure is in line with the current hypothesis of prenatal and/or early-life origin of TGCT. The limitations of the studies may partly explain the inconsistencies observed. Unfortunately, we were unable to perform a quantitative meta-analysis because of the heterogeneity in design, exposure assessment and population characteristics for the studies we identified. However, many risk factors investigated in current studies remain of interest, in particular pesticides, and recent studies highlight the potential role of gene-environment interactions. Further large studies are needed, and future research should focus on prenatal or early life exposure, as well as combined effect of prenatal and adolescent or adulthood exposure.

Supporting Information

Table S1. Occupational and environmental exposure related testicular germ cell cancer (publication from 1990 to 2012).
(DOCX)

Checklist S1. Completed PRISMA Checklist.
(DOC)

Acknowledgements

Thanks to Niels Erik Skakkebaek of Rigshospitalet (Copenhagen, DENMARK), to Aude Flechon and Helen Boyle of the Centre Léon Bérard (Lyon, France), and to Helen Bailey of the International Agency for Research on Cancer (Lyon, France) for their helpful advice and assistance in the preparation of our manuscript. In addition, we would like to acknowledge editorial assistance from Margaret Haugh (MediCom Consult).

Author Contributions

Performed the experiments: RB CLC. Analyzed the data: RB CLC JS BF. Contributed reagents/materials/analysis tools: RB CLC. Wrote the manuscript: RB CLC. Arbitration during the appraisal of the studies methodology: JS BF. Article revision and supervision: JS BF.

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Supplemental Materials

Occupational and environmental exposures associated with testicular germ cell tumours: systematic review of prenatal and life-long exposures

Rémi Béranger, Charlotte Le Cornet, Joachim Schüz, Béatrice Fervers

Table S1: Occupational and environmental exposure related testicular germ cell cancer (publication from 1990 to 2012)

Exposures	Ref.	All testicular cancer	Seminoma	Non-seminoma
EXPOSURES TO OCCUPATIONS AND TASKS				
PLASTIC INDUSTRY				
Plastics workers	(Ohlson and Hardell 2000)	OR = 3.3 [1.4-7.7]	-	-
Worker exposed to plastics	(Hansen 1999)	OR = 0.9 [0.6-1.2]	-	-
Manufacture of plastics	(Walschaerts et al. 2007)	OR = 2.87 [0.84–9.77]	-	-
Rubber, plastic products industry	(Van den Eeden et al. 1991)	RR = 0.6 [0.1-5.7]	-	-
Vinyl monochloride (VMC) or polyvinyl chloride (PVC)				
VMC	(Langard et al. 2000)	1 Obs / 0.75 Exp [0.03-7.43]	-	-
PVC	(Ohlson and Hardell 2000)	OR = 6.6 [1.4-32]	OR = 5.6 [1.1-196]	-
PVC	(Hansen 1999)	OR = 0.4 [0.1-1.0]	-	-
PVC (1 year latency)	(Hardell et al. 2004)	OR = 1.35 [1.06-1.71]		
PVC (10 year latency)	(Hardell et al. 2004)	OR = 1.45 [1.06-1.98]	-	-
METAL INDUSTRY				
Metalworkers	(Rhomberg et al. 1995)	OR = 1.2 [0.78-1.84]	OR = 2.04 [1.13-3.68]	OR = 0.8 [0.47-1.34]
Metal products industry	(Knight et al. 1996)	-	OR = 0.49 [0.22-1.09]	-
Metal products industry	(Van den Eeden et al. 1991)	RR = 2.0 [1.0-3.8]	-	-
By specific tasks				
Welding	(Walschaerts et al. 2007)	OR = 2.84 [1.51-5.35]		

Welding[a]	(Walschaerts et al. 2007)	OR = 1.49 [0.53-4.15][a]	-	-
Welding	(Ohlson and Hardell 2000)	OR = 0.9 [0.5-1.6]	-	-
Welding	(Hansen et al. 1996)	SIR = 0.80 [0.47-1.26]	-	-
Foundry work	(Walschaerts et al. 2007)	OR = 0.90 [0.25–3.22]	-	-
High dermal exposure with oil-based metal working fluids	(Behrens et al. 2012)	OR = 1.87 [1.05-3.34]	OR = 1.28 [0.60-2.73]	OR = 4.72 [1.48-15.09]
Metal trimming	(Walschaerts et al. 2007)	OR = 1.49 [0.53–4.15]	-	-
Metal annealer, temperer	(Pollan et al. 2001)	-	RR = 5.85 [1.88-18.20]	-
Stainless steel grinders	(Hansen et al. 1996)	SIR = 2.41 [0.97-4.97]	-	-
Furnace workers in a ferrosilicon plant	(Hobbesland et al. 1999)	SIR = 2.3 [1.05-4.37]		
Non furnace workers in a ferrosilicon plant	(Hobbesland et al. 1999)	SIR = 0.72 [0.2-1.85]		
PAPER INDUSTRY				
Pulp and paper maintenance workers	(Andersson et al. 2003)	SIR = 4.8 [1.3-12]	SIR = 6.6 [1.8-17]	-
Paper and printing workers	(Swerdlow et al. 1991)	OR = 2.05 [0.84-5.02]	-	-
Paper products industry	(Van den Eeden et al. 1991)	RR = 0.8 [0.3-2.3]	-	-
Printing, publishing industry	(Van den Eeden et al. 1991)	RR = 1.0 [0.4-2.5]	-	-
Pulp or paper dust	(Guo et al. 2005)	OR = 2.19 [0.06–12.2]	-	-
Paper mill workers employees	(Rix et al. 1998)	SIR = 1.04 [0.70-1.49]	-	-
Pulp and paper mill workers1	(Band et al. 2001)	SIR = 0.96 [0.66-1.36]	-	-
Sulphate workers (<10y)	(Andersson et al. 2012)	SIR = 1.87 [1.00-3.20]	-	-
Sulphate workers (≥10 y)	(Andersson et al. 2012)	SIR = 0.84 [0.31-1.83]	-	-
Sulphite workers (<10y)	(Andersson et al. 2012)	SIR = 1.12 [0.36-2.60]	-	-
WHITE COLLAR				
Professionals (administrator, teacher, physician, veterinarian)	(Hayes et al. 1990)	OR = 1.0 [0.7-1.6]	OR = 2.8 [1.4-5.4]	OR = 0.7 [0.5-1.2]
Administrators, manager	(Van den Eeden et al. 1991)	RR = 1.5 [1.1-2.2]	-	-
Administrators	(Swerdlow et al. 1991)	OR = 1.33 [0.74-2.42]		
Administrative and managerial	(Pollan et al. 2001)	-	RR = 1.20 [0.83-1.73]	RR = 1.75 [1.09-2.82]
Professional and technical work	(Pollan et al. 2001)	-	RR = 1.06 [0.89-1.26]	RR = 1.15 [0.91-1.46]
Engineer, architect, surveyor	(Van den Eeden et al. 1991)	RR = 0.8 [0.4-1.6]		
Engineering workers	(Swerdlow et al. 1991)	OR = 0.73 [0.52-1.03]		
Amusement and recreation worker	(Knight et al. 1996)	-	OR = 2.15 [1.17-3.95]	-
Business services	(Knight et al. 1996)	-	OR = 1.66 [1.04-2.63]	
Health related	(Hayes et al. 1990)	OR = 1.4 [0.7-2.6]	OR = 1.9 [0.7-5.0]	OR = 1.2 [0.6-2.5]

Physician & other health-related jobs	(Van den Eeden et al. 1991)	RR = 5.5 [1.1-26.3]	-	-
School teacher	(Pollan et al. 2001)	-	RR = 2.30 [1.19-4.43]	RR = 1.35 [0.43-4.20]
University, high education teacher	(Pollan et al. 2001)	-	RR = 1.83 [0.91-3.69]	-
Journalist, editor	(Pollan et al. 2001)	-	RR = 2.57 [1.22-5.42]	-
Government legislative administrator	(Pollan et al. 2001)	-	RR = 1.96 [1.05-3.66]	-
Bookkeeping and clerical work	(Pollan et al. 2001)	-	RR = 1.28 [0.95-1.72]	RR = 1.65 [1.14-2.38]
Professional vs manual worker	(Swerdlow et al. 1991)	OR = 1.99 [1.14-3.47]		
CONSTRUCTION WORKERS				
Wood workers - carpenters				
Carpenter	(Dement et al. 2003)	SIR = 1.29 [0.78-2.01]	-	-
Carpenter (15y lag from entry date in the union)	(Dement et al. 2003)	SIR = 2.48 [1.29-4.32]	-	-
Carpenter	(Van den Eeden et al. 1991)	RR = 1.2 [0.7-2.0]	-	-
Construction	(Hayes et al. 1990)	OR = 0.8 [0.6-1.2]	OR = 0.4 [0.2-0.9]	OR = 1.0 [0.7-1.5]
Construction carpenters	(Guo et al. 2005)	OR = 0.4 [0.1–1.1]	-	-
Woodworkers	(Swerdlow et al. 1991)	OR = 0.74 [0.35-1.53]	-	-
Lumber, wood products	(Van den Eeden et al. 1991)	RR = 0.8 [0.5-1.4]	-	-
Wood dust	(Guo et al. 2005)	OR = 0.81 [0.26–1.89]	-	-
Electrical workers				
Electrical workers	(Swerdlow et al. 1991)	OR = 0.74 [0.40-1.37]	-	-
Electrician occupation	(Van den Eeden et al. 1991)	RR = 2.8 [1.2-6.4]	-	-
Electrical workers	(Tynes et al. 1992)	SIR = 0.83 [0.59-1.12]	-	-
Electrician	(Ohlson and Hardell 2000)	OR = 1.0 [0.4-2.6]	-	-
Utilities in electrical power industry	(Knight et al. 1996)	-	-	OR = 3.15 [1.15-8.61]
Painters				
Painter occupation	(Van den Eeden et al. 1991)	RR = 1.3 [0.6-3.2]	-	-
Industrial paints occupation	(Walschaerts et al. 2007)	OR = 0.63 [0.18–2.25]	-	-
Painters and decorators	(Swerdlow et al. 1991)	OR = 0.83 [0.35-1.97]	-	-
FIREMEN				
Between 1977 and 1996	(Bates et al. 2001)	SIR = 1.55 [0.8-2.8]	-	-
Between 1990 and 1996	(Bates et al. 2001)	SIR = 2.97 [1.3-5.9]	-	-
Between 1988 and 2003	(Bates 2007)	OR = 1.54 [1.18-2.02]	-	-
Between 1988 and 1995	(Bates 2007)	OR = 1.92 [1.32-2.80]	-	-
Between 1996 and 2003	(Bates 2007)	OR = 1.29 [0.87-1.92]	-	-
Fire brigade members	(Giles et al. 1993)	SIR = 1.15 [0.13-4.17]	-	-
Ever worked as firefighter	(Stang et al. 2003)	OR = 4.5 [0.7-31.9]	-	-
Ever worked as firefighter	(Ma et al. 2006)	SIR = 1.6 [1.2-2.09]	-	-

POLICEMEN				
	(Davis and Mostofi 1993b)	SIR = 6.9 (p<0.001)	-	-
	(Finkelstein 1998)	OR = 1.33 [90%CI 1.0-1.75]	-	-
	(Pollan et al. 2001)	-	RR = 1.85 [1.05-3.28]	-
	(Guo et al. 2004)	SIR = 1.10 [0.23-3.22]	-	-
MILITARY PERSONNEL				
Service in royal navy	(Ryder et al. 1997)	OR = 1.08 [0.51-2.28]	-	-
Services and military work	(Pollan et al. 2001)	-	RR = 1.42 [1.08-1.86]	RR = 0.98 [0.63-1.54]
Armed forces	(Swerdlow et al. 1991)	OR = 0.84 [0.53-1.33]	-	-
Vietnam service	(Tarone et al. 1991)	OR = 2.5 [1.1-5.7]	OR = 1.8 [0.6-5.1]	OR = 2.4 [1.1-5.4]
Air force				
Air force versus Army	(Bullman et al. 1994)	OR = 1.48 [0.62-3.48]	-	-
Air force versus Army	(Knoke et al. 1998)	OR = 1.28 [1.02-1.62]	-	-
Royal Air Force	(Foley et al. 1995)	OR = 3.27 [2.43-4.31]	-	-
Air force (Vietnam veterans)	(Tarone et al. 1991)	OR = 1.8 [0.1-117.8]	-	-
Air force (total flight hours ≥1)	(Yamane and Johnson 2003)	OR = 1.74 [1.04–2.92] / but no association when stratified by time		
Aviator vs non flying officer	(Grayson and Lyons 1996)	SIR = 1.84 [99%CI 1.19-2.86]		
Aviator vs general population	(Grayson and Lyons 1996)	SIR = 1.04 [99%CI 0.72-1.44]		
Aircrew (air force & civil)	(Milanov et al. 1999)	SIR = 2.51 [0.90-4.92]		
Air force	(Yamane 2006)	SIR = 0.68 [0.61-0.75]		
Marine - navy				
Navy vs Army	(Bullman et al. 1994)	OR = 2.60 [1.08-6.24]	-	-
Navy vs Army	(Knoke et al. 1998)	OR = 1.29 [1.03-1.62]	-	-
Navy (Vietnam veterans)	(Tarone et al. 1991)	OR = 3.4 [0.6- 23.8]	-	-
Marine	(Bullman et al. 1994)	OR = 0.46 [0.19-1.07]	-	-
Marines versus Army	(Knoke et al. 1998)	OR = 1.00 [0.71-1.41]	-	-
Marines in Vietnam veterans	(Tarone et al. 1991)	OR = 0.7 [0.1-7.9]	-	-
Royal marines	(Ryder et al. 1997)	OR = 0.79 [0.89-1.64]	-	-
Coast guard vs Army	(Knoke et al. 1998)	OR = 1.31 [0.74-2.32]	-	-
Others				
Balkans mission	(Gustavsson et al. 2004)	SIR = 1.9 [0.8-3.7]	-	-
Fleet air arm vs others branches	(Ryder et al. 1997)	OR = 1.90 [1.04-3.48]	-	-
Engineer air vs other specialties	(Ryder et al. 1997)	OR = 2.32 [1.20-4.48]	-	-
Engineer air handler vs others	(Ryder et al. 1997)	OR = 7.31 [1.81-29.53]	-	-
>3 days of agent orange spaying within 2km	(Bullman et al. 1994)	OR = 1.39 [0.50-3.80]	-	-

>90 days of agent orange spaying within 8km	(Bullman et al. 1994)	OR = 0.99 [0.54-1.84]	-	-
AGRICULTURAL WORKERS AND PESTICIDES USERS				
Agriculture/ forestry/ fishing	(Hayes et al. 1990)	OR = 0.9 [0.6-1.4]	OR = 0.4 [0.2-0.9]	OR = 1.1 [0.7-1.8]
Agriculture/ forestry/ fishing	(Pollan et al. 2001)	-	RR = 1.04 [0.78-1.38]	RR = 0.84 [0.55-1.28]
Agriculture/ forestry/ fishing	(Swerdlow et al. 1991)	OR = 1.09 [0.65-1.83]	-	-
Agricultural workers	(Pollan et al. 2001)	-	-	RR = 1.17 [0.52-2.64]
Agricultural workers	(Guo et al. 2005)	OR = 1.0 [0.4–2.0]	-	-
Farm manager	(Van den Eeden et al. 1991)	RR = 1.9 [0.6-5.4]	-	-
Farm worker and gardener	(Van den Eeden et al. 1991)	RR = 0.6 [0.3-1.3]	-	-
Farmers	(Kelleher et al. 1998)	SIR = 0.65 [0.26-1.33]	-	-
Other agriculture (fish farmers)	(Kelleher et al. 1998)	SIR = 3.77 [1.03-9.67]	-	-
Pesticides industry	(Walschaerts et al. 2007)	OR = 0.86 [0.28–2.66]	-	-
Licensed pesticide applicators	(Dich et al. 1996)	SIR = 1.09 [0.68-1.67]	-	-
Licensed pesticide applicators	(Frost et al. 2011)	SIR = 1.26 [1.04-1.53]	-	-
Licensed pesticide applicators	(Fleming et al. 1999)	SIR = 2.48 [1.57-3.72]	-	-
Commercial pesticide applicators	(Alavanja et al. 2005)	SIR = 1.24 [0.33-3.17]	-	-
Pesticides industry	(Walschaerts et al. 2007)	OR = 0.86 [0.28–2.66]	-	-
OTHER OCCUPATIONAL EXPOSURES				
Chemicals industry	(Van den Eeden et al. 1991)	RR = 1.3 [0.5-3.6]	-	-
Chemicals industry	(Walschaerts et al. 2007)	OR = 1.57 [0.74–3.33]	-	-
Nitrate fertilizer workers	(Zandjani et al. 1994)	SIR = 226 [91-465]	-	-
Food and beverage processors	(Knight et al. 1996)	-	-	OR = 3.20 [1.39-7.35]
Food products manufacture industry	(Van den Eeden et al. 1991)	RR = 2.2 [1.0-4.9]	-	-
Leather products industry	(Knight et al. 1996)	-	-	OR = 4.60 [0.75-28.28]
Leather dyeing and tanning	(Knight et al. 1996)	-	-	OR = 4.21 [1.25-14.13]
Leather workers	(Marshall et al. 1990)	OR = 7.2 [1.89-27.72]	-	-
Leather workers	(Swerdlow et al. 1991)	OR = 1.05 [0.33-3.42]	-	-
Leather dust	(Guo et al. 2005)	OR = 6.77 [0.17–37.7]	-	-
Deck officer	(Sulem and Rafnsson 2003)	SIR = 0.0[0.0-0.7]		
Radiologic technologists	(Sigurdson et al. 2003)	SIR = 1.32[0.76-2.13]		
EXPOSURES TO RADIATIONS				
MAGNETIC & ELECTRIC FIELDS				
General				
Working near radiofrequency emitters	(Baumgardt-Elms et al. 2002)	OR = 0.9 [0.60–1.24]	No difference observed	

Working near electrical machines	(Baumgardt-Elms et al. 2002)	OR = 1.0 [0.72–1.33]	No difference observed	
Working near high-voltage lines	(Baumgardt-Elms et al. 2002)	OR = 0.7 [0.38–1.18]	No difference observed	
Working in front of a visual display unit or in complex electrical environments	(Baumgardt-Elms et al. 2002)	OR = 0.9 [0.67–1.21]	No difference observed	
High-voltage lines (<100 m)	(Baumgardt-Elms et al. 2005)	OR = 1.5 [0.66-3.43]	-	-
High-voltage lines (>26 days)	(Baumgardt-Elms et al. 2005)	OR = 1.8 [0.91-3.38]	-	-
Working near radar units	(Baumgardt-Elms et al. 2002)	OR = 1.0 [0.60–1.75]	No difference observed	
Radar equipment	(Hayes et al. 1990)	OR = 1.1 [0.7-1.9]	OR = 1.3 [0.6-2.8]	OR = 1.1 [0.6-1.9]
Occupation related to radar	(Walschaerts et al. 2007)	OR = 0.84 [0.38–1.87]	-	-
Microwaves or radio waves	(Hayes et al. 1990)	OR = 0.8 [0.3-2.0]	-	-
Magnetic fields (using JEM)				
Medium level	(Floderus et al. 1999)	RR = 1.6 [1.2-2.1]	-	-
High level		RR = 1.7 [1.3-2.2]		
≤40y in 90th percentile	(Stenlund and Floderus 1997)	OR = 2.8 [1.1-6.9] (P90)	OR = 1.5 [0.5-5.0]	OR = 4.6 [1.5-13.6]
≤40y in 4th quartile		OR = 1.8 [0.9-3.4] (Q4)	OR = 1.1 [0.5-2.5]	OR = 2.9 [1.2-7.0]
IONIZING RADIATION				
Radioactive material	(Hayes et al. 1990)	OR = 1.2 [0.46-2.3]	OR = 1.3 [0.5-3.3]	OR = 1.2 [0.6-2.4]
Nuclear activity	(Walschaerts et al. 2007)	OR = 2.13 [0.85–5.37]	-	-
EXPOSURE TO CHEMICALS				
PESTICIDES – GENERAL				
Pesticides	(Guo et al. 2005)	OR = 1.28 [0.55–2.53]	-	-
Pesticides	(Hayes et al. 1990)	OR = 1.2 [0.4-1.2]	OR = 0.1 [0.0-1.0]	OR = 1.5 [0.9-2.7]
Pesticides	(Swerdlow et al. 1991)	OR = 1.04 [0.61-1.77]	-	-
Private pesticide use	(Alavanja et al. 2005)	SIR = 1.05 [0.67-1.58]	-	-
Pesticide or herbicide spraying	(Knight et al. 1996)	-	OR = 0.63 [0.42-0.95]	-
Mixing pesticides for gardening	(Giannandrea et al. 2011)	OR = 4.80 [0.91-25.30]	-	-
Use of pesticide for gardening	(Nori et al. 2006)	OR = 1.22 [0.60-2.48]	OR = 0.55 [0.18-1.61]	OR = 2.23 [0.97-5.10]
Exposed to herbicides	(Guo et al. 2005)	OR = 1.00 [0.68–1.43]	-	-
Exposed to herbicides	(Swerdlow et al. 1991)	OR = 1.14 [0.67-1.94]	-	-
Exposed to fungicides	(Guo et al. 2005)	OR = 1.04 [0.75–1.41]	-	-
Exposed to insecticides	(Guo et al. 2005)	OR = 3.05 [0.83–7.81]	-	-

Exposed to insecticides	(Giannandrea et al. 2011)	OR = 3.23 [1.15-9.11]	-	-
PESTICIDES – SERUM CONCENTRATIONS				
β-hexachlorocyclohexane	(McGlynn et al. 2008)	OR = 0.90[0.65-1.24]	OR = 0.97 [0.63-1.49]	OR = 0.85 [0.57-1.26]
	(Biggs et al. 2008)	OR = 0.92 [0.51-1.64]	-	-
γ-hexachlorocyclohexane	(Biggs et al. 2008)	OR = 1.36 [0.75-2.46]	-	-
Dieldrin	(Biggs et al. 2008)	OR = 0.79 [0.44-1.41]	-	-
Hexachlorobenzene (HCB)	(Biggs et al. 2008)	OR = 0.85 [0.37-1.96]	-	-
Heptachlor epoxide	(Biggs et al. 2008)	OR = 0.67 [0.35-1.29]	-	-
p,p'-DDT	(Biggs et al. 2008)	OR = 1.17 [0.68-2.00]	-	-
	(McGlynn et al. 2008)	OR = 1.13 [0.71-1.82]	OR = 1.30 [0.73-2.30]	OR = 0.94 [0.50-1.77]
	(Purdue et al. 2009)	OR = 2.1 [0.6-7.2]	-	-
o,p'-DDT	(Biggs et al. 2008)	OR = 1.30 [0.67-2.53]	-	-
	(Purdue et al. 2009)	OR = 1.4 [0.4-4.5]	-	-
p,p'-DDE	(Biggs et al. 2008)	OR = 0.61 [0.32-1.14]	-	-
	(Giannandrea et al. 2011)	OR = 3.21 [0.77-13.30]	-	-
	(McGlynn et al. 2008)	OR = 1.71 [1.23-2.38]	OR = 1.91 [1.22-2.99]	OR = 1.63 [1.10-2.42]
	(Purdue et al. 2009)	OR = 2.2 [0.7-6.5]	OR = 2.2 [0.5-8.7]	-
Oxychlordane	(Biggs et al. 2008)	OR = 0.93 [0.50-1.73]	-	-
	(McGlynn et al. 2008)	OR = 1.27 [0.92-1.76]	OR = 1.64 [1.04-2.60]	OR = 1.11 [0.75-1.63]
	(Purdue et al. 2009)	OR = 3.2 [0.6-16.8]	OR = 5.1 [0.7-36.8]	-
cis-nonachlor	(McGlynn et al. 2008)	OR = 1.56 [1.11-2.18]	OR = 1.93 [1.27-2.93]	OR = 1.32 [0.86-2.03]
Mirex	(McGlynn et al. 2008)	OR = 1.24 [0.90-1.74]	OR = 1.15 [0.75-1.77]	OR = 1.24 [0.82-1.88]
	(Purdue et al. 2009)	OR = 1.2 [0.4-3.0]	-	-
Trans-nonachlor	(Biggs et al. 2008)	OR = 0.89 [0.49-1.61]		
	(McGlynn et al. 2008)	OR = 1.46 [1.07-2.00]	OR = 1.72 [1.11-2.67]	OR = 1.39 [0.96-2.00]
	(Purdue et al. 2009)	OR = 2.6 [0.7-8.9]	OR = 1.6 [0.4-6.0]	-
Trans-nonachlor + Gene CYP1A1 polymorphisms rs7495708	(Chia et al. 2010a)	OR = 1.92 [1.03–3.58]	-	
Trans-nonachlor + Gene CYP1A1 polymorphisms rs1456432	(Chia et al. 2010a)	OR = 1.90 [1.01–3.56]	-	
Total chlordanes	(McGlynn et al. 2008)	OR = 1.51 [1.09-2.10]	OR = 1.90 [1.20-3.00]	OR = 1.37 [0.93-2.02]
	(Biggs et al. 2008)	OR = 0.93 [0.51-1.68]		
	(Purdue et al. 2009)	OR = 2.3 [0.6-7.2]	OR = 1.6 [0.4-6.6]	-
Total chlordanes + gene CYP1A1 polymorphisms rs7495708	(Chia et al. 2010a)	OR = 2.21 [1.17–4.15]	-	-
Total chlordanes + gene CYP1A1 polymorphisms rs1456432	(Chia et al. 2010a)	OR = 2.07 [1.09–3.92]	-	-

Total organochlorine pesticides (HCB+p,p'-DDE)	(Giannandrea et al. 2011)	OR = 3.34 [1.09-10.17]	-	-
CHLORINATED BIPHENYLS				
PCB 99	(McGlynn et al. 2009)	OR = 0.80 [0.57-1.13]	OR = 0.80 [0.51-1.25]	OR = 0.76 [0.50-1.17]
	(Purdue et al. 2009)	OR = 2.2 [0.8-5.9]	OR = 4.4 [1.0-20.5]	-
PCB 101	(McGlynn et al. 2009)	OR = 1.01 [0.74-1.38]	OR = 1.12 [0.74-1.70]	OR = 0.91 [0.62-1.33]
PCB-118	(McGlynn et al. 2009)	OR = 0.55 [0.40-0.76]	OR = 0.72 [0.47-1.12]	OR = 0.45 [0.31-0.66]
PCB-118 + gene HSD17B4 polymorphisms rs384346	(Chia et al. 2010a)	OR = 0.46 [0.31–0.70]	Not shown	
PCB-138	(McGlynn et al. 2009)	OR = 0.46 [0.32-0.66]	OR = 0.52 [0.31-0.86]	OR = 0.42 [0.27-0.65]
PCB-138	(Purdue et al. 2009)	OR = 1.8 [0.6–5.1]	OR = 2.1 [0.6-7.2]	-
PCB-138 + Gene HSD17B4 polymorphisms rs384346	(Chia et al. 2010a)	OR = 0.46 [0.30–0.72]	Not shown	
PCB 153	(McGlynn et al. 2009)	OR = 0.45 [0.31-0.66]	OR = 0.52 [0.31-0.87]	OR = 0.40 [0.26-0.63]
	(Purdue et al. 2009)	OR = 1.2 [0.4-3.4]	OR = 1.2 [0.4-4.3]	-
PCB 156	(McGlynn et al. 2009)	OR = 0.57 [0.40-0.81]	OR = 0.54 [0.34-0.86]	OR = 0.58 [0.37-0.91]
PCB 163	(McGlynn et al. 2009)	OR = 0.59 [0.42-0.83]	OR = 0.58 [0.37-0.92]	OR = 0.57 [0.37-0.86]
PCB 167	(Purdue et al. 2009)	OR = 4.4 [1.0-19.8]	OR = 6.7 [1.1-42.9]	-
PCB 170	(McGlynn et al. 2009)	OR = 0.56 [0.39-0.80]	OR = 0.56 [0.35-0.91]	OR = 0.55 [0.36-0.85]
PCB 180	(McGlynn et al. 2009)	OR = 0.56 [0.38-0.82]	OR = 0.67 [0.39-1.13]	OR = 0.51 [0.32-0.81]
PCB 183	(McGlynn et al. 2009)	OR = 0.86 [0.58-1.29]	OR = 0.77 [0.46-1.29]	OR = 0.92 [0.56-1.52]
	(Purdue et al. 2009)	OR = 1.3 [0.5-3.5]	OR = 2.9 [0.6-13.7]	-
PCB 187	(McGlynn et al. 2009)	OR = 0.60 [0.42-0.86]	OR = 0.75 [0.47-1.20]	OR = 0.48 [0.31-0.75]
Sum of PCBs (99, 101, 118, 138, 153, 156, 163, 170, 180, 183, 187)	(McGlynn et al. 2009)	OR = 0.46 [0.32-0.67]	OR = 0.45 [0.27-0.76]	OR = 0.45 [0.29-0.71]
Sum of PCB (31 congeners)	(Purdue et al. 2009)	OR = 1.3 [0.5-3.8]	OR = 1.2 [0.4-4.1]	-
SOLVENTS				
Formaldehyde	(Guo et al. 2005)	OR = 1.03 [0.28–2.64]	-	-
HYDROCARBONS				
Diesel	(Guo et al. 2004)	OR = 1.15[0.36-3.60]	-	-
Diesel exhaust	(Guo et al. 2005)	OR = 1.20 [0.67–1.98]	-	-
Gasoline exhaust	(Guo et al. 2004)	OR = 1.58 [0.22-11.4]	-	-
Gasoline engine exhaust	(Guo et al. 2005)	OR = 1.02 [0.44–2.01]	-	-
Gasoline	(Guo et al. 2005)	OR = 1.19 [0.44–2.59]	-	-
Polycyclic aromatic hydrocarbons/ combustion/ drilling of fossil fuels	(Hayes et al. 1990)	OR = 1.5 [0.7-3.4]	OR = 1.0 [0.2-3.9]	OR = 1.7 [0.7-4.0]

Petroleum/coal refining & products industry	(Van den Eeden et al. 1991)	RR = 1.1 [0.2-7.3]	-	-
OTHER EXPOSURES				
TEMPERATURE				
Extreme (<60 F° or >80 F°)	(Zhang et al. 1995)	OR = 1.71 [1.13-2.60]	-	-
Low temperature (<60 F°)	(Zhang et al. 1995)	OR = 1.70 [1.04-2.78]	-	-
High temperature (>80F °)	(Zhang et al. 1995)	OR = 1.20 [0.80-1.80]	-	-
RESIDENCY				
Adulthood				
Urban vs rural	(Ohlson and Hardell 2000)	OR = 1.5 [0.9-2.4]	-	-
Rural vs urban	(Nori et al. 2006)	OR = 1.27 [0.32-5.05]	OR = 1.40 [0.27-7.09]	OR = 1.58 [0.26-9.75]
Rural vs urban	(Walschaerts et al. 2007)	OR = 1.63 [1.16-2.29]	-	-
Rural vs urbain[a]	(Walschaerts et al. 2007)	OR = 1.43 [0.83–2.46][a]	-	-
very dense urbanization (≤2500 hab/km^2) vs. rest of the Netherlands	(Sonneveld et al. 1999)	IR = 4.4 (NS)[b]	IR = 2.3 (NS)[b]	IR = 1.8 (NS)[b]
Very low urbanization (<500 hab/km^2) vs. rest of the Netherlands	(Sonneveld et al. 1999)	IR = 4.4 (NS)[b]	IR = 2.3 (NS)[b]	IR = 1.8 (NS)[b]
Living in area exposed to metals and PCBs	(Helmfrid et al. 2012)	SIR = 2.46 [0.99-2.42]	-	-
Childhood - Adolescence				
Urban vs rural (childhood)	(Ohlson and Hardell 2000)	OR = 1.3 [0.8-2.0]	-	-
Childhood in area with ≥ 6 fungal warning a year	(Kristensen et al. 2000)	RR = 1.2[0.7-2.1]	-	-
Childhood in the country	(Moller 1997)	OR = 0.79 [0.63-1.00]	OR = 0.85 [0.63-1.13]	OR = 0.71 [0.52-0.98]
Childhood in high-nitrate area	(Moller 1997)	OR = 1.40 [1.09-1.81]	OR = 1.36 [1.00-1.86]	OR = 1.49 [1.06-2.08]
Rural vs urban at adolescence	(Nori et al. 2006)	OR = 5.73 [1.26-25.97]	OR = 12.14 [2.14-68.78]	OR = 1.47[0.18-11.81]
More than 6 month in a farm	(Moller 1997)	OR = 0.72 [0.55-0.94]	OR = 0.86 [0.62-1.19]	OR = 0.57 [0.39-0.83]

Abbreviations: y = year; JEM = job exposure matrix; DDT = dichlorodiphenyltrichloroethane; DDE= Dichlorodiphenyldichloroethylene.
a: Adjusted for environmental and occupational exposures and reproductive health history.
b: Association was declared to be not significant but confidence interval was not shown.
If not specified, confidence interval (IC) is 95%.

Chapter III:
Environmental exposures to pesticides

Development of a geographical approach to assess environmental exposure to agricultural pesticides in France

III.1 Synthèse en français / summary in English

SYNTHESE – FRANCAIS

Objectif du chapitre : Le développement d'une nouvelle approche GIS visant à évaluer les expositions environnementales aux pesticides agricoles en France, nécessaire dans le cadre de notre projet, nécessitait la réalisation d'une étude complémentaire. D'après la littérature, la poussière domestique était une matrice pertinente pour évaluer l'exposition des ménages aux pesticides des ménages, et ainsi identifier les déterminants de l'exposition environnementale aux pesticides agricoles. Dans un premier temps, nous avons testé la capacité d'une lingette en cellulose à collecter la poussière domestique et les pesticides y étant adsorbés. Ensuite, nous avons réalisé une campagne de prélèvements domestiques en région Rhône-Alpes pour caractériser l'exposition aux pesticides de foyers proches de différents types de cultures. A partir de ces résultats, nous avons identifié les déterminants de l'exposition environnementale aux pesticides agricoles.

Validation de la lingette : Initialement, la lingette en cellulose utilisée pour les prélèvements de sols et de poussières anciennes a été choisie après concertation avec Rovaltain Research Company (anciennement « plateforme de toxicologie et d'écotoxicologie de Rovaltain »). Notre critère principal était la capacité de la lingette à collecter les poussières, dans la mesure où les pesticides sont facilement adsorbés sur les particules de poussières domestique. Une série de blancs ont été fait pour vérifier l'absence de contamination intrinsèque des lingettes, ainsi qu'un douzaine de prélèvements de poussières domestiques en Rhône-Alpes pour vérifier notre capacité à détecter des pesticides (43 pesticides distincts ont été retrouvés). Toutefois, l'utilisation de lingettes en cellulose n'a jamais été validée dans ce contexte, et nous n'avions aucune idée de l'efficacité et de la précision de cette lingette concernant la collecte de pesticides.

Nous avons donc étudié la précision et l'efficacité de la lingette en cellulose concernant la collecte de 48 pesticides, huit PCBs et un synergiste pour pesticide (piperonyl butoxide ; PB). Une première expérience visait à déterminer l'efficacité et la répétabilité

de la lingette lorsque la solution était directement déposée sur trois types de surface fréquemment retrouvés dans les foyers (carrelage, parquet stratifié et parquet contrecollé). Une deuxième expérience visait à vérifier la capacité de notre lingette à collecter de la poussière synthétique seule déposée sur du carrelage. Dans la troisième expérience, les composés étaient adsorbés sur la poussière synthétique, puis la poussière dopée était déposée sur du carrelage pour être ensuite essuyée à l'aide de notre lingette. Dans la première expérience, la récupération moyenne était meilleure pour le carrelage et le parquet stratifié que pour le parquet contrecollé (38%, 40% et 34%, respectivement ; $p<0.001$). La deuxième expérience a confirmé que la lingette en cellulose permettait de collecter efficacement la poussière synthétique seule (82% d'efficacité). La troisième expérience a montré que la récupération moyenne de pesticides et PCBs est statistiquement plus importante en présence de poussière synthétique (72% vs. 38% sans poussières ; $p<0.001$). La répétabilité moyenne était également améliorée en présence de poussière synthétique (<30% pour la majorité des composés). A notre connaissance, notre étude est la première à tester l'efficacité d'une lingette pour collecter des pesticides en se basant sur une sélection de composés aussi large, à concentrations environnementales, et en présence de poussières. La lingette en cellulose apparait donc efficace pour prélever les pesticides et PCBs adsorbés sur de la poussière, sur des surface dures et lisses.

Prélèvements de poussières et analyses : Nous avons échantillonné 239 foyers de la région Rhône-Alpes (France) en 2012 : 69 à proximité de cultures arboricoles, 66 à proximité de cultures céréalières, 68 à proximité de cultures viticoles et 36 en milieux urbain (à 2000m minimum de toutes cultures). Les agriculteurs et les applicateurs de pesticides professionnels n'étaient pas inclus dans l'étude. Pendant la période principale d'application de pesticides de chaque secteur, nous avons réalisé des prélèvements de poussière à l'aide de pièges à poussières (30 jours d'accumulation passive de poussières) et de lingettes en cellulose imbibées d'isopropanol (prélèvements de sol : 7 jours d'accumulation ; prélèvement de rebords de fenêtres ou de portes d'entrées : au moins 6 mois d'accumulation). Les caractéristiques du foyer et les utilisations potentielles de pesticides en milieu domestique ont été recueillies à l'aide d'un questionnaire administré. Nous avons analysé les poussières en laboratoire par une approche multirésidue

(chromatographie liquide et gazeuse) permettant la détection de 417 composés (406 pesticides, 10 métabolites et le PB). Les prélèvements de poussières récentes (RDS : prélèvements de sols et pièges à poussières) visaient à mesurer l'exposition actuelle du foyer, alors que les poussières anciennes (ODS : rebords de portes ou fenêtres) visaient à mesurer l'exposition cumulée, dans la mesure où les pesticides sont stables dans la poussière domestique.

Caractérisation de l'exposition des foyers : le PB ainsi que 156 pesticides ont été détectés au moins une fois dans l'ensemble des foyers, mais à des taux de détection faible dans la plupart des cas. En croisant les données provenant du Ministère de l'Agriculture français, des Chambres Départementales de l'Agriculture (DAC), des vendeurs de pesticides, de fermiers des secteurs concernés, et des foyers participants, nous avons regroupé les pesticides en fonction de leur utilisation en 2012 ; agricole, domestique, ou interdite. En se focalisant sur les RDS, 1327 détections ont été observées (pour 120 pesticides). Les composés interdits représentaient 32% des détections, les composés à usage domestique exclusif 28%, les composés à usage mixte agricole et domestique 24%, et les composés à usage agricole exclusif 16%. Une utilisation domestique de pesticides est rapportée pour 87% des foyers, ce qui est en accord avec le fort taux de détection de composés à usage domestique. Nos résultats confirment de précédents travaux suggérant que les foyers proches de cultures agricoles sont contaminés par des pesticides à usage agricole. Toutefois, la forte prévalence de composés à usage domestique ou interdits suggère que ces sources d'expositions doivent être prises en compte dans les futures études épidémiologiques afin de limiter le risque de biais de classement. La présence de composés interdits dans les RDS suggère une contamination actuelle. Une utilisation de ces composés interdits est possible, mais il semble plus probable que l'origine de ces expositions soit liée à une réémission à partir de l'environnement ou de sources domestiques (ex : matériaux de construction, sols…).

Déterminent environnementaux de l'exposition : à partir d'un GIS, nous avons définis pour chaque foyer la surface totale de culture en se basant sur cinq tailles de buffer différentes (250m, 500m, 750m, 1000m, et 1250m), l'impact des vents dominants, et la

présence de barrières végétales, topographiques et structurelles. Afin d'exprimer l'impact des vents dominants et des barrières, nous avons développé une nouvelle approche appelée CAP (Contributive Area for Pesticide drift). Les pesticides agricoles autorisés pour la culture d'intérêt et détectés dans plus de 10% des foyers du secteur considéré ont été inclus dans les analyses statistiques (Analyses De Redondance (RDA) – approche à la fois multivariable et multivariée). La taille du buffer optimal varie selon le type de culture observé (500m pour les vergers, 1000m pour les vignes et les céréales). D'une manière générale, les déterminants de la contamination des poussières domestiques aux pesticides agricoles sont similaires selon les différentes approches testées. La surface des cultures à l'intérieur des buffers, les vents dominants et la présence de barrière végétales apparaissent comme les principaux déterminants de l'exposition. La variabilité expliquée par nos modèles reste modeste (7.1 – 18.3%), mais conforme aux précédent résultats de la littérature. L'approche utilisée pour caractériser l'impact des vents dominants et des barrières apparait comme prometteuse pour de futures études. Ces résultats serviront de base pour la création d'une nouvelle métrique, adaptée au contexte français, pour l'évaluation des expositions environnementales aux pesticides agricoles.

SUMMARY - ENGLISH

Aim of the chapter: While Geographical Information Systems (GIS) have been proposed to reliably characterize environmental pesticide exposures, further research was needed to develop, improve and validate a new GIS metric in this area, in particular in the French context. Based on the literature, indoor dust sampling has been suggested as a valid approach to estimate households' exposures and to serve as basis for identifying the determinants of the households' environmental exposure to agricultural pesticides originating from the surrounding environment. Our ability to collect and detect dust and pesticides using cellulose wipes has been assessed in a methodological study conducted in laboratory. Based on a survey of indoor dust sampling in the Rhône-Alpes region (France), we characterized indoor pesticide contaminations of households, separately for areas with different land use. Based on these results, we identified determinants of the agricultural pesticide exposures for the different land use.

Wipe validation: Initially, the cellulose wipes used for floor wipe and window/door edge samples were chosen after consultation with the Rovaltain Research Company (formerly: Rovaltain Research Facility for Environmental Toxicology and Ecotoxicology). Our main criterion was the ability of the wipe to collect dust, since pesticides are easily adsorbed on indoor dust particles. Several blanks were made to ensure the absence of contamination and 12 samples were taken in four households of the Rhône-Alpes area to test our ability to detect pesticides (43 pesticides have been detected). However, the use of cellulose wipe has never been validated in previous studies, and we had no idea of the exact wipe collection efficiency and the repeatability for pesticides.

We assessed the efficiency and precision of a cellulose wipe for collecting 48 pesticides, eight PCBs and one pesticide synergist (piperonyl butoxide; PB) at environmental concentrations. In a first experiment, the efficiency and repeatability of wipe collection were determined for pesticide and PCB residues that were directly spiked onto three types of household floors (tile, laminate, and hardwood). In a second experiment, synthetic dust alone was used to assess the capacity of the wipe to collect dust. Then, for the third experiment, we assessed the efficiency and repeatability of wipe collection of pesticides and PCBs residues that were spiked onto synthetic dust and then applied to tile. In the first experiment, overall collection efficiency was higher on tile (38%) and laminate (40%) compared to hardwood (34%), $p<0.001$. The second experiment confirmed that cellulose wipes can efficiently collect dust (82% collection efficiency). The third experiment showed that overall collection efficiency was higher in the presence of dust (72% vs. 38% without dust, $p<0.001$). Mean repeatability was greatly improved when compounds were spiked onto dust (<30% for the majority of compounds). To our knowledge, this study is the first to assess efficiency of wipes as a sampling method using a large number of organic compounds at environmental concentrations and synthetic dust. Cellulose wipes appear to be efficient to sample pesticides and PCBs adsorbed onto dust on smooth and hard surfaces.

Dust collection and laboratory analyses: We sampled 239 households in the Rhône-Alpes region (France) in 2012: 69 in orchards production area, 66 near corns and grains, 68 near vineyards and 36 houses in an urban area (at least 2000 meters from agricultural fields). Homes of farmers and of pesticides applicators have been excluded from the study. During the main period of pesticide application, we used a polypropylene dust trap (30 days of passive dust accumulation) and cellulose wipes moistened with isopropanol, to collect dust on the floor (7 days of accumulation) and on window sills or the edge of the entrance door (at least 6 month). Household characteristics and related domestic pesticide use were assessed by questionnaire. We conducted multi-residue laboratory analyses for 417 compounds (406 pesticides, 10 metabolites, and PB) using gas and liquid chromatography. Recent dust samples (RDS) from dust trap and floor wipe samples were considered to reflect current exposures, while old dust samples (ODS) from window sills or door edges were considered to represent a household's cumulative exposure because pesticides remain stable on indoor dust particles.

Characterization of the indoor contamination: PB and 156 pesticides were detected at least once in all households, but at a low detection rate for the majority of them. By synthesizing the data from the French Ministry of Agriculture, the Departmental Agricultural Chambers (DAC), pesticides vendors, local farmers, and study households, we defined pesticides that were authorized in agriculture, used for domestic purpose, as well as banned pesticides (for 2012). In the RDS, 1327 detections were observed (120 pesticides and PB). Banned pesticides represented 32% of pesticides detected; pesticides used for domestic purposes only 28%; pesticides having both domestic and agricultural use 24%; and pesticides restricted to agricultural use represented 16%. In 87% of households, domestic pesticide use was reported, which was in line with the high detection rate of domestic pesticides. Our results confirmed previous work suggesting indoor contamination by agricultural pesticides in households close to agricultural settings. However, the high prevalence of domestic and banned compounds suggested that these exposure sources should be considered in future epidemiological studies to avoid potential misclassification bias. Interestingly, we detected banned pesticides in RDS, indicating on-going contamination by these pesticides. This might result from

continued use, or more likely from continuous reemission from environmental or domestic sources (e.g. from construction materials or soils).

Environmental determinants of agricultural pesticide exposure: Using a GIS, we defined for each residence the total acreage of crops for five different buffer sizes (250m, 500m, 750m, 1000m, and 1250m), the prevailing winds, and the presence of vegetative, topographic and structural barriers. For each study households, prevailing winds and barriers were taken into account using a new approach called "*contributive area for pesticide drifts*" (CAP). Agricultural pesticides authorized for the targeted crop type and detected in more than 10% of study households were considered in our statistical analyses using redundancy analyses (multivariate and multivariable models). Optimal buffer size varied depending on the type of crops observed (500m for orchards, 1000m for vineyards and corn/grain). Overall, determinants of agricultural pesticide concentrations in indoor dust were consistent between approaches tested. Crop acreage, prevailing winds, and presence of vegetative barriers appeared to be the main determinants observed across models. Overall, variability explained by the models remained modest (7.1–18.3%), but was consistent with the literature. Approaches developed to assess the impact of the wind and of the presence of barriers are promising areas for future study. These results will provide a basis for developing a new GIS metric, adapted to the French context, for assessing environmental exposures to agricultural pesticides.

III.2 (article #2)

Efficiency of wipe sampling on hard surfaces for pesticides and PCBs residues in house dust

Joane Cettier [(1)$], Marie-Laure Bayle [(2)$], Rémi Béranger [(1, 3, 4)], Elise Billoir [(2)], John R. Nuckols [(5)], Bruno Combourieu [(2)£], Béatrice Fervers[(1)£]

(1) Unit of Cancer and Environment, Centre Léon Bérard, 28 rue Laënnec, Lyon, France

(2) Rovaltain Research Company, 1 rue de la gare, Alixan, Valence, France

(3) Section of Environment and Radiation, International Agency for Research on Cancer, 150 cours Albert Thomas, Lyon, France

(4) EAM 4128 « Santé Individu Société », University Claude Bernard, 43 boulevard du 11 novembre 1918, Villeurbanne, France

(5) Department of Environmental and Radiological Health Sciences, Colorado State University, Fort Collins, CO, United State

[$]The first two authors contributed equally to this work and share first authorship

[£]The last two authors contributed equally to this work and share last authorship

Article submitted to *Science of the Total Environment*

(In Press)

III.2.1 Introduction

Pesticides and polychlorinated biphenyls (PCBs) have been extensively used worldwide since the 1930s and are found in all environmental compartments (water, soil, and air). Human exposure to these molecules is associated with several diseases, including cancer, Parkinson's disease, and infertility (Bretveld et al., 2007; Brody et al., 2004; Lauby-Secretan et al., 2013; McGlynn et al., 2008; Noyce et al., 2012; Provost et al., 2007). These semi-volatile organic compounds have been shown to easily bind to particles such as dust (Weschler and Nazaroff, 2008). Therefore, the surface dislodgeable residues found in homes (SDRs; often named "housedust" or "indoor dust" in the literature) are a repository of pesticides and other chemicals used indoors or adsorbed on particles from the outside (Mercier et al., 2011; Obendorf et al., 2006). Several studies have shown the presence of pesticides and PCBs in SDR samples (Butte and Heinzow, 2002; Colt et al., 2004; Golla et al., 2012; Harrad et al., 2009; Julien et al., 2007; Knobeloch et al., 2012; Obendorf et al., 2006; Quiros-Alcala et al., 2011; Stout II et al., 2009).

Pesticides detected in SDRs usually originate from indoor use and transport of lawn and garden chemicals (Lewis et al., 1999), as well as from nearby agricultural fields (Gunier et al., 2011; Ward et al., 2006). Collection of SDR samples is a relatively cheap and straightforward method to determine the level of indoor contamination from organic compounds (Butte and Heinzow, 2002; Lioy et al., 2002; Mercier et al., 2011). The mean environmental load of SDRs varies across studies: 0.01 (p,p'-dichlorodiphenyldichloroethylene) to 2.9 ng/cm² (cypermethrin) in the study by Stout II et al. (2009), 0.0002 (malathion) to 0.061 ng/cm² (*t*-permethrin) in the study by Clifton et al. (2013), and from 0.004 (*t*-mevinphos) to 0.42 ng/cm² (chlorpyrifos) in the study by Tulve et al. (2006). In a previous French study, the median load of compounds using wipes was 0.02–0.04 ng/cm² on average, depending on the agricultural area (Béranger et al., 2013).

By contrast, PCBs found in SDRs usually arise from building and decorating materials, such as paints, wood floor finishes and caulking, adhesives, tapes, plastics, and thermal

insulation (Knobeloch et al., 2012; Mercier et al., 2011; USEPA, 2013). Previous studies have shown that PCB contamination in SDRs is 9.2–290 ng/g on average in various areas of the world (Harrad et al., 2009) and 8.8–1,186.5 ng/g according to the time period and the American counties (Knobeloch et al., 2012).

Various methods have been used to collect SDRs from carpets, including a High Volume Small Surface Sampler (HSV3) (Golla et al., 2012; Quiros-Alcala et al., 2011), commercial vacuum (Harrad et al., 2009; Obendorf et al., 2006), and samples from personal vacuum bags (Colt et al., 2004; Knobeloch et al., 2012). Although less frequently used for the assessment of organic contaminants, wipes represent an alternative active method to sample SDRs. This method consists of wiping a delimited sampling area using a wipe wetted with a solvent, usually isopropanol or a mixture of methanol and distilled water (Mercier et al., 2011). Wipes are more commonly used for hard surfaces (Stout II et al., 2009; Julien et al., 2007). Wipes were preferred in large-scale studies for its ease of use and its absence of intrinsic contamination (Deziel et al., 2011; Mercier et al., 2011), whereas vacuums are thought expensive and cumbersome. Additionally, fine particles could be resuspended during vacuum sampling, and the finest particles contain most pesticides (Lewis et al., 1999; Lioy et al., 2002).

Several studies have shown that the efficiency of wipe collection for organic chemical residues is influenced by the surface material (Bernard et al., 2008; Carr and Hill, 1989; Madireddy et al., 2013; Nussbaumer et al., 2012; Sottani et al., 2007; Willison, 2012), as well as the type of wipe employed and solvent used as the wetting agent (Carr and Hill, 1989; Deziel et al., 2011; Nussbaumer et al., 2012; Willison, 2012). However, validation studies using wipes for sampling pesticides residues are sparse, and the existing publications vary in design, limiting comparability. Bernard et al. (2008) tested the collection efficiency of wipes wetted with isopropanol or a Press Sampler on different surfaces. The authors concluded that wipes wetted with a solvent removed all tested pesticides and were more efficient than the Press Sampler on hard surfaces. Deziel et al. (2011) studied the impact of various solvents and wipes on the collection efficiency for pesticides on a stainless steel surface. Better overall collection efficiencies were found

when Twillwipes (cotton wipe) were wetted with isopropanol compared to Twillwipes wetted with deionised water and polyvinyl alcohol wipes wetted with deionised water (Deziel et al., 2011). A study was conducted in 1989 by the United States Environmental Protection Agency (USEPA) (Carr and Hill, 1989). The best recovery for PCBs (Arochlor 1260) was found with paper wipes (saturation pads compared to polyurethane foam plugs, cotton balls and dental wicks) and isooctane as the solvent (compared to acetone, isopropanol and dichloromethane). To our knowledge, no study investigates the efficiency of wipe collection of organic compounds adsorbed on SDR, or the collection efficiency of cellulose wipes to sample organic compounds.

The aim of our study was to assess, in a controlled laboratory environment, the accuracy and precision of cellulose wipes for 48 pesticides, one pesticide synergist, and eight PCBs applied directly to hard floor surfaces or adsorbed onto synthetic dust. We further assessed the association between the physicochemical properties of the compounds and the collection efficiency of the wipes.

III.2.2 Materials and Methods

a) Study design

We assessed the collection efficiency (accuracy of the method) and repeatability (precision of the method) of wipe sampling on a 40 cm × 40 cm (1600 cm^2) test surface using a solution of 57 compounds (48 pesticides, one pesticide synergist, and eight PCBs) solubilised in acetone (Table 3.1). Three experiments were conducted: 1) direct application of the Pesticides-PCBs solution to three different test surfaces (tile, laminate, and hardwood); 2) application of synthetic dust to the three test surfaces; and 3) application of synthetic dust contaminated with the Pesticides-PCBs solution to the tile surface. Table 3.2 summarises the three experiments. One technician conducted the experiments using the same laboratory and building materials and the same procedure in the same laboratory over a short period of time.

Table 3.1: Concentrations of positive controls, experimental collection efficiency and repeatability for all compounds in experiments one and three

		Experiment one								Experiment three						
		Positive controls (µg/L)	Experimental samples							Positive controls (µg/L)		Experimental samples				
			Tile		Laminate		Hardwood			Without dust	With dust	Without dust		With dust		
Substance group	Compound	Mean ± SD	Collection efficiency %	Repeatability %	Collection efficiency %	Repeatability %	Collection efficiency %	Repeatability %	p-value[£]	Mean ± SD	Mean ± SD	Collection efficiency %	Repeatability %	Collection efficiency %	Repeatability %	p-value[¥]
Organochlorine	PCB-101	35 ± 10	52	28	52	30	38	25	<0.05	35 ± 10	46 ± 5	52	28	76	18	<0.01
Organochlorine	PCB-105	47 ± 12	42	32	39	28	28	23	<0.01	47 ± 12	57 ± 7	42	32	66	18	<0.01
Organochlorine	PCB-118	46 ± 10	44	28	40	33	30	16	<0.05	46 ± 10	64 ± 8	44	28	63	18	<0.01
Organochlorine	PCB-138	48 ± 11	43	32	43	30	29	17	<0.01	48 ± 11	53 ± 7	43	32	61	19	<0.01
Organochlorine	PCB-153	42 ± 10	44	28	43	32	29	25	<0.01	42 ± 10	59 ± 7	44	28	61	17	<0.01
Organochlorine	PCB-180	47 ± 11	45	28	41	25	29	20	<0.01	47 ± 11	55 ± 2	45	28	61	21	<0.05
Organochlorine	PCB-28	13 ± 7	52	32	60	43	44	45	NS	13 ± 7	25 ± 4	52	32	95	17	<0.001
Organochlorine	PCB-52	20 ± 11	28	22	40	40	32	44	NS	20 ± 11	26 ± 5	28	22	87	17	<0.001
Organochlorine	Lindane	8 ± 5	47	28	41	84	25	53	<0.01	8 ± 5	17 ± 5	47	28	81	25	<0.001
Organochlorine	Pentachlorophenol	12 ± 4	27	100	19	139	27	122	NS	12 ± 4	14 ± 3	27	100	101	14	<0.001
Triazole	Azaconazole	21 ± 7	23	63	22	64	16	238	<0.05	21 ± 7	18 ± 5	23	63	66	31	<0.001
Triazole	Cyproconazole	23 ± 6	28	54	33	21	21	82	<0.05	23 ± 6	15 ± 3	28	54	65	32	<0.001
Triazole	Difenoconazole	14 ± 4	11	156	11	180	0	*	NS	14 ± 4	16 ± 3	11	156	65	28	<0.001
Triazole	Fenbuconazole	21 ± 5	29	44	27	55	31	66	NS	21 ± 5	18 ± 4	29	44	73	32	<0.001
Triazole	Myclobutanil	33 ± 7	48	23	44	17	34	44	<0.05	33 ± 7	20 ± 6	48	23	68	30	<0.05
Triazole	Tebuconazole	20 ± 5	45	155	34	21	9	139	<0.001	20 ± 5	18 ± 3	45	155	66	33	<0.01
Organophosphate	Azinphos-methyl	23 ± 8	25	65	16	103	12	118	NS	23 ± 8	21 ± 8	25	65	80	35	<0.001
Organophosphate	Chlorpyrifos	14 ± 7	55	33	63	37	51	29	NS	14 ± 7	24 ± 5	55	33	78	28	<0.001
Organophosphate	Chlorpyrifos-methyl	7 ± 3	55	67	78	63	89	34	<0.05	7 ± 3	14 ± 2	55	67	89	29	<0.001
Organophosphate	Diazinon	6 ± 4	46	46	64	42	34	95	<0.05	6 ± 4	8 ± 2	46	46	79	26	<0.001
Pyrethroid	Cypermethrin	84 ± 69	31	92	49	68	68	68	NS	84 ± 69	40 ± 2	31	92	67	42	<0.01
Pyrethroid	Deltamethrin	24 ± 5	100	57	70	29	66	120	<0.05	24 ± 5	30 ± 4	100	57	5	288	<0.001

Pyrethroid	λ-Cyalothrin	38 ± 9	43	36	47	27	28	23	<0.01	38 ± 9	26 ± 2	43	36	66	29	<0.001
Pyrethroid	Permethrin	37 ± 13	56	26	61	27	49	25	NS	37 ± 13	28 ± 4	56	26	66	19	<0.05
Carbamate	Chlorpropham	8 ± 3	50	30	59	30	66	38	NS	8 ± 3	12 ± 2	50	30	88	26	<0.001
Carbamate	Dimetilan	13 ± 4	0	*	0	*	0	*	$	13 ± 4	18 ± 3	0	*	78	20	<0.01
Carbamate	Propoxur	16 ± 7	32	29	28	49	27	30	NS	16 ± 7	11 ± 4	32	29	96	23	<0.001
Chloroacetamid	Acetochlor	23 ± 7	38	31	42	33	37	19	NS	23 ± 7	13 ± 8	38	31	93	29	<0.001
Chloroacetamid	Dimethenamid-P	14 ± 5	48	22	47	32	61	25	<0.05	14 ± 5	7 ± 4	48	22	85	61	<0.05
Chloroacetamide	Metolachlor	42 ± 11	40	33	45	28	37	19	NS	42 ± 11	28 ± 5	40	33	68	27	<0.001
Morpholine	Dimethomorph	29 ± 8	28	38	29	18	44	133	NS	29 ± 8	19 ± 6	28	38	60	46	<0.01
Morpholine	Spiroxamine	27 ± 11	73	27	75	21	53	15	<0.01	27 ± 11	24 ± 18	73	27	111	31	<0.05
Strobilurin	Kresoxim methyl	42 ± 9	42	42	47	57	75	136	NS	42 ± 9	34 ± 4	42	42	110	164	<0.05
Strobilurin	Trifloxystrobin	40 ± 14	42	33	44	34	29	41	<0.05	40 ± 14	32 ± 3	42	33	52	32	<0.05
Anylinopyrimidine	Pyrimethanil	15 ± 5	31	26	30	36	25	32	NS	15 ± 5	8 ± 5	31	26	79	57	<0.05
Benzoylurea	Flufenoxuron	42 ± 7	50	34	61	17	32	21	<0.001	42 ± 7	33 ± 9	50	34	91	36	<0.01
Cyanoacetamide oxime	Cymoxanil	13 ± 5	4	380	0	*	0	*	NS	13 ± 5	12 ± 5	4	380	54	43	<0.001
Dinitroanilinine	Pendimethalin	24 ± 11	35	63	56	39	52	31	<0.05	24 ± 11	36 ± 6	35	63	52	53	<0.01
Dinitrophenol	DNOC	7 ± 4	0	*	0	*	8	350	NS	7 ± 4	15 ± 10	0	*	121	51	<0.001
Neonicotinoid	Imidacloprid	7 ± 5	0	*	0	*	0	*	$	7 ± 5	16 ± 4	0	*	53	33	<0.001
Phenol	Orthophenylphenol	10 ± 5	44	26	45	81	38	45	NS	10 ± 5	16 ± 6	44	26	80	38	<0.001
Phenylpiridinamine	Fluazinam	23 ± 5	37	36	39	28	17	82	<0.001	23 ± 5	22 ± 2	37	36	66	17	<0.001
Phenylpyrazole	Fipronil	31 ± 10	29	29	32	23	24	15	<0.05	31 ± 10	29 ± 2	29	29	20	49	<0.05
Phenylpyrrole	Fludioxonil	30 ± 10	40	31	44	48	37	30	NS	30 ± 10	46 ± 5	40	31	65	19	<0.001
Phenylurea	Diuron	16 ± 5	8	192	3	300	0	*	NS	16 ± 5	12 ± 7	8	192	71	53	<0.01
Phtalimide	Folpet	77 ± 27	47	32	67	39	52	38	NS	77 ± 27	67 ± 9	47	32	52	30	<0.05
Piperonyl	Piperonyl butoxide	33 ± 10	43	33	46	39	38	22	NS	33 ± 10	34 ± 3	43	33	67	30	<0.05
	Overall/Mean	27 ± 17	38	55	40	50	34	62		27 ± 17	27 ± 16	38	55	72	39	

SD: Standard Deviation; NS: Non-significant ($p \geq 0.05$).

*: repeatability could not be calculated because collection efficiency was equal to 0.

$: statistics could not be performed for the compound because the collection efficiencies were non-quantified (replaced by 0)

£: Comparison of the collection efficiency by surface type (Kruskal-Wallis test)

¥: Comparison of the collection efficiency in absence and presence of dust (Wilcoxon-Mann-Whitney test)

Nine pesticides were excluded from the analyses and were not presented in this table: anthraquinone; azinphos-ethyl; bifenthrin; dicamba; diflubenzuron; iodocarb; mesotrione; propiconazole; and sulcotrione.

Table 3.2: Summary characteristics for each experiment

	Experiment No. 1	Experiment No. 2	Experiment No. 3
Groups Number	three (surfaces)	three (surfaces)	two (dust absence/dust presence)
Floors	tile, laminate, hardwood	tile, laminate, hardwood	tile
Concentration	50 µg/L (S_1)	-	250 µg/L (S_2)
Solution volume	1 mL	-	200 µL
Dust	-	100 mg	100 mg
Contamination	spiked on the surfaces	-	adsorbed on dust
Number of replicates	17 (tile)*, 11 (laminate and hardwood)	11	17
Number of samples	39	33	34

*: The same 17 replicates without dust in experiment three correspond to the 17 replicates applied to tile from experiment one.

b) Pesticides and PCBs tested

The 57 organic compounds were selected based on the contaminants found in the SDRs of 239 houses in a companion study performed in the Rhône-Alpes region (France) (Béranger et al., 2013) and pesticides reported by French local agricultural agencies as commonly used in the main crops in the Rhône-Alpes region (orchards, cereals, and vineyards) (personal communications). For each compound, we identified the physicochemical properties (log Kow [octanol-water partition coefficient], log Koa [octanol-air partition coefficient], log Koc [organic carbon sorption constant] and solubility in water) based on five online databases: PesticidePropertiesDataBase (University of Hertfordshire, 2014), ChemIDplus (United States National Library of Medicine, 2014), AGRITOX (ANSES, 2014), ChemSpider (Royal Society of Chemistry, 2014), and International Chemical Safety Cards (NIOSH and Institut Scientifique de la Santé Publique, 2006).

c) Solution preparation

The Pesticides-PCBs spiking solution was prepared in acetone (99.8% purity, Carlo Erba Reagents; Milano, Italy) in order to obtain a mixture of 57 compounds, corresponding to a load of 0.03 ng/cm² (solution S_1; average concentration: 55µg/L, see Supplemental Materials, Table S1). This is close to the pesticide surface loadings previously reported from indoor floor wipe dust samples (Béranger et al., 2013; Clifton et al., 2013; Stout II et al., 2009; Tulve et al., 2006). An additional solution (solution S_2) was prepared for experiment three by concentrating the solution S_1 by a factor of five under a nitrogen stream. The solution of pesticides and PCBs used was prepared from pure individual standard solutions in acetone (Techlab; Metz, France/Sigma-aldrich; Saint-Quentin Fallavier, France/LGC Standards; Molsheim, France). All solutions were stored in a glass bottle at -20°C.

d) Household floors

Our study was conducted using three types of floor (tile, laminate and hardwood); these were the most commonly found floors in French homes in the study by Béranger et al. (2013). The tile (glazed porcelain stoneware) consisted of a smooth, porous, non-permeable material with a waterproof coating on the surface. The laminate consisted of a kraft paper impregnated with melamine resin. It had a lightly rough, non-porous, slightly permeable surface. The hardwood consisted of a piece of glazed wood that had a rough, porous, permeable surface. The sampling surfaces are shown in the supplemental materials (Supplemental Materials; Figure S1). The laminate and hardwood floors were cut to 45 cm × 45 cm, and the tile was directly bought in a 45 cm × 45 cm size, providing a central test surface of 40 cm × 40 cm that was bordered by an engrave in each corner. The laminate and hardwood floors were screwed onto a 50 cm × 50 cm Plexiglas plate, and the tile was stuck to the Plexiglas using a double-sided adhesive (Goman 24, Uzin;

Ulm, Germany). The floors were thoroughly cleaned with deionised water and isopropanol (99.9% purity, Fischer Scientific; Waltham, MA, USA) prior to the experiments and between each replicate.

e) Synthetic dust

To simulate housedust, we used ASHRAE 52/76 test dust (Particle Technology; Derbyshire, Hatton, England). ASHRAE synthetic dust contains 23% black carbon (particle size 2.6–13.2 μm), 72% mineral dust (<80 μm), and 5% cotton linters. This synthetic dust, which has a standardised composition and particle size, was chosen to limit variability across the experiments. For experiments two and three, we used 100 mg of dust per sample, which approximately corresponds to one month of dust deposition in a 1 m² section of home (Edwards et al., 1998).

f) Wipes and solvent

We used 11 cm × 21 cm cellulose wipes (KimtechScience, reference 7552, Kimberly-Clark Professional; Irving, Texas, USA). To remove any contaminants, all wipes were pre-cleaned by placing twenty wipes in a separating funnel with 150 mL of dichloromethane (99.9% purity, Carlo Erba Reagents; Milano, Italy). After shaking for 1 min, the wipes were dried under fume hood airflow. The wipes were then placed in a sealed glass jar until use. For each surface sampling, two wipes were saturated with 10 mL of isopropanol in a Pyrex beaker and agitated until they were soaked. As done in previous studies (Billets, 2008; Willison, 2012), the first wipe was used to wipe the 40 cm × 40 cm test area, by drawing a Z-shape, making small circles from left to right, top to bottom, and then placed into a sterile Pyrex Erlenmeyer flask with a glass stopper. The same procedure was used for the second wipe, except the movement was right to left, bottom to top. This second wipe was placed in the same flask and was stored under dark conditions at 4°C until extraction (within 24 h).

g) Determination of the number of replicates

Replicates were defined as repeated samplings within the same experimental condition. For each experiment, the number of replicates was determined to reach a power of 80% for a one-factor variance analysis, with an alpha risk of 5% and an effect size of 0.5. This resulted in 11 replicates for each of the three surface types for experiments one and two and 17 replicates for each of the two conditions (presence and absence of dust) in experiment three.

h) Experiment one: wiping of pesticides and PCBs directly applied to test surfaces

Experiment one assessed the efficiency and the repeatability of wipe collection for sampling pesticides and PCBs directly applied to the three test surfaces (tile, hardwood, and laminate). For each replicate, 1,000 µL of solution S_1 was applied in ten droplets to the test area. After the droplets dried (about 30 s), the surface was wiped (Section 2.6). For the laminate and hardwood, 11 replicates were performed. For the tile, 17 replicates were performed to allow comparison with experiment three (see below). In total, 39 samples were performed.

i) Experiment two: wiping of synthetic dust on test surfaces

Experiment two assessed the efficiency and repeatability of wipe collection for sampling dust on the three test surfaces (i.e. no spiking solution added). Using a precision balance (max = 210 g, d = 0.1 mg, Mettler Toledo; Viroflay, France), 100 mg of ASHRAE test dust was weighed onto an aluminium foil. Then, the operator gently shook the aluminium foil to distribute the dust onto the sampling area. The operator visually ensured that all of the dust had been scattered from the foil to the surface (the dust consisted of easily visible

dark small particles). Each group of two wipes was weighed before and after wiping to determine the mass of the collected dust. Eleven replicates were performed for each of the three test surfaces (33 samples in total). Additionally, five positive controls (direct deposit of 100 mg synthetic dust onto the wipes) were performed.

j) Experiment three: wiping of synthetic dust contaminated with pesticides and PCBs on tile

Experiment three compared the efficiency and repeatability of wipe collection for sampling pesticides and PCBs directly applied to tile (corresponding to the 17 replicates from experiment one) or adsorbed onto synthetic dust. To mimic adsorption, the synthetic dust was spiked with solution S_2. One hundred milligrammes of synthetic dust was weighed onto an aluminium foil and contaminated with 200 µL of solution S_2, to obtain the same quantity of pesticides and PCBs as in the 1000 µL of S_1 used in experiment one. The contaminated synthetic dust was kept in the fume hood for 10–15 min at ambient temperature in order to allow solvent evaporation and compound adsorption. The contaminated synthetic dust was then scattered on the tile (see Section 2.9) and wiped, according to the standard procedure described in Section 2.6. Seventeen replicates were performed and compared to the 17 replicates made on tile without dust (34 samples in total).

k) Extraction and analysis of compounds

After sampling the pesticides and PCBs (experiments one and three), the flasks were opened in a fume hood for 10 h to allow evaporation of the isopropanol before the extraction process. Each sample was spiked with 100 µL of extraction internal standards, which were composed of hexabromobenzene (2 mg/L) and triphenylphosphate (10 mg/L) (Restek; Bellefonte, PA, USA). The pesticides and PCBs extraction consisted of adding 150 mL of dichloromethane (99.9% purity, Carlo Erba Reagents; Milano, Italy) into the

flasks; the flasks were then stoppered and placed on a platform shaker for 4 h. The flask's contents were filtered with a funnel filled with glass wool containing sodium sulphate powder (99% purity, Chemlab; Zedelgen, Belgium). One millilitre of isooctane (99.5% purity, Carlo Erba Reagents; Milano, Italy) was added to the filtrate, which had been concentrated to a final volume above 500 µL in a TurboVap® evaporator workstation under nitrogen stream at 35°C (TurboVap® II Zymark, Sotax; Allschwil, Switzerland). The volume was then adjusted to 1 mL by adding ethyl acetate (99.8% purity, Carlo Erba Reagents; Milano, Italy). From this, 450 µL was collected, evaporated to dryness, and then resuspended in 450 µL of water and acetonitrile (1:1), which had been acidified with 0.1% formic acid. This solution was analysed using high performance liquid chromatography (HPLC) (Agilent 1100, Agilent Technologies; Waldbronn, Germany) coupled with a tandem mass spectrophotometer (MS/MS) (API4000, AB Sciex; Foster City, CA, USA).

A purification step was carried out on 500 µL of the remaining extract using magnesium silicate cartridges (CHROMABOND® Florisil®, Macherey-Nagel; Düren, Germany) with methanol (99.9% purity, Carlo Erba; Milano, Italy) for column conditioning, and hexane (95% purity, Carlo Erba Reagents; Milano, Italy) and ethyl acetate as elution solvents. The purified filtrate was then concentrated in a TurboVap® to a final volume of 500 µL; from this, 450 µL was collected in a vial. Chrysene-D12 was added as an internal standard, and the sample was analysed using gas chromatography (GC) (Varian-GC 450, SGE, Ringwood; Victoria, Australia) coupled with mass spectrophotometer (MS) (Varian Saturn 2000, SGE, Ringwood; Victoria, Australia).

Analyses were performed with the analytical conditions listed in the supplemental materials (Supplemental Materials; Table S2) and in accordance with ISO 17025 guidelines (UNIDO, 2009). All compounds were quantified using a calibration range from 1 to 100 µg/L. The limit of quantification (LQ) was 1 µg/L for each compound. For all samples analysed, the extraction of internal standards was quantified for results acceptance. Hexabromobenzene, chrysene-D12 (for GC-MS), and triphenylphosphate (for HPLC-MS/MS) were all within a 20% variation range of the expected concentration.

Solvents, pre-cleaned wipes, and synthetic dust were analysed with GC-MS and HPLC-MS/MS (full scan and multiple reaction monitoring (MRM) modes) and showed no intrinsic contamination, except for propiconazole in dust (this compound was excluded from the statistical analysis). Additionally, two negative controls and five positive controls were performed for each experimental condition. The negative controls in experiment one consisted of wiping sampling surfaces that were spiked with pure acetone: one before each series of replicates (to check for intrinsic contamination) and one at the middle of the series (to check for potential accumulation). In experiment three, the negative controls consisted of depositing uncontaminated dust on tile. The positive controls consisted in applying the spiking solution (experiment one) or the spiked dust (experiment three) directly on the wetted wipes, without surface wiping. Concentrations of the negative controls are detailed in the supplemental materials (Supplemental Materials; Table S1) and results of the positive controls are presented in Table 3.1.

l) Collection efficiency and repeatability

Of the 57 compounds tested in the current study, nine pesticides were excluded from further analysis: (i) two (azinphos-ethyl and bifenthrin) because the mean individual concentrations were below the negative controls, (ii) two (anthraquinone and propiconazole) because the mean individual concentrations were above the sum of the mean and standard deviation of the positive controls, and (iii) five (dicamba, diflubenzuron, iodocarb, mesotrione, and sulcotrione) because they were not quantified in the positive controls. Data analysis was performed on 39 pesticides, eight PCBs, and one pesticide synergist.

The collection efficiency of the wipes in experiments one and three was calculated using the formula below, which was adapted from USEPA (Billets, 2008) by replacing the initial concentration with the positive control concentration in the denominator to overcome the impact of extraction and analytical methods on the performance of our

wipe method and estimate the faction of pesticides transferred and wiped (Table 3.1) (Bernard et al., 2008).

$$\text{Collection efficiency}_{\text{compound}}(\%) = \frac{C_{\text{measured}}}{C_{\text{positive control}}} \times 100$$

where $C_{measured}$ is the mean individual concentration of a given compound across the replicates and $C_{positive\ control}$ the mean individual concentration of the same compound across the positive controls. Values below the LQ were replaced by 0 (worst case). For the remaining 48 compounds, the overall (i.e. all compounds combined) collection efficiency was calculated by averaging the individual compound collection efficiencies for each experimental condition.

The collection efficiency for experiment two was calculated using the following formula:

$$\text{Collection efficiency}_{\text{dust}}(\%) = \frac{(M_{\text{after}} - M_{\text{wipe}})}{M_{\text{dust}}} \times 100$$

where M_{after} is the mean mass of the wipe after dust sampling, M_{wipe} the mean mass of the wipes before sampling, and M_{dust} the mean dust mass deposited on the surface.

We calculated the precision of the method by its repeatability, which is expressed as the relative standard deviation (RSD) using the formula below (Billets, 2008):

$$\text{RSD}\ (\%) = \left|\frac{SD}{\bar{C}}\right| \times 100$$

where $\bar{C}$ is the mean concentration for a given compound across the replicates and SD the standard deviation. The mean repeatability was calculated by meaning individual RSDs for each experimental condition.

m) Statistical analysis

Using the Shapiro-Wilk test, we found that the data on collection efficiencies were not normally distributed (test applied on individual results for each compound). Therefore, we used the Wilcoxon-Mann-Whitney (two groups) or Kruskal-Wallis (more than two groups) non-parametric tests to compare the collection efficiencies between the types of surface (experiments one and two), and between the presence and absence of dust (experiment three). The comparison tests were performed for all compounds combined, as well as for each compounds individually. The Bonferroni correction was applied to the alpha risk (5%) to correct for multiple statistical analyses.

A multivariable analysis (i.e. considering a multivariate response) of all compounds was performed on the collection efficiency results from experiments one and three using redundancy analysis (RDA), a method that combine regression and principal component analysis. This method is an alternative to MANOVA (multi-response ANOVA), which relaxes the assumption of multivariate normality of each group of data (Borcard et al., 2011). This analysis tested the relationship of multiple linear regression between a response matrix Y and an explanatory variables matrix X (Borcard et al., 2011). In our case, matrix Y was the efficiency of the wipe collection for the different compounds ([log + 1] transformed), and matrix X was the factor of interest: surface type for experiment one and the presence/absence of dust for experiment three. The significance of the relationship was evaluated with a permutation test, as explained in Borcard et al. (2011).

To examine the correlation between the collection efficiencies and physicochemical properties of the compounds (log Kow, log Koc, log Koa and solubility in water) in experiments one and three, we used the Spearman's rank correlation test. All statistics were performed using the R software (v 2.15.3). The limit of significance was set as 5% (p-value < 0.05).

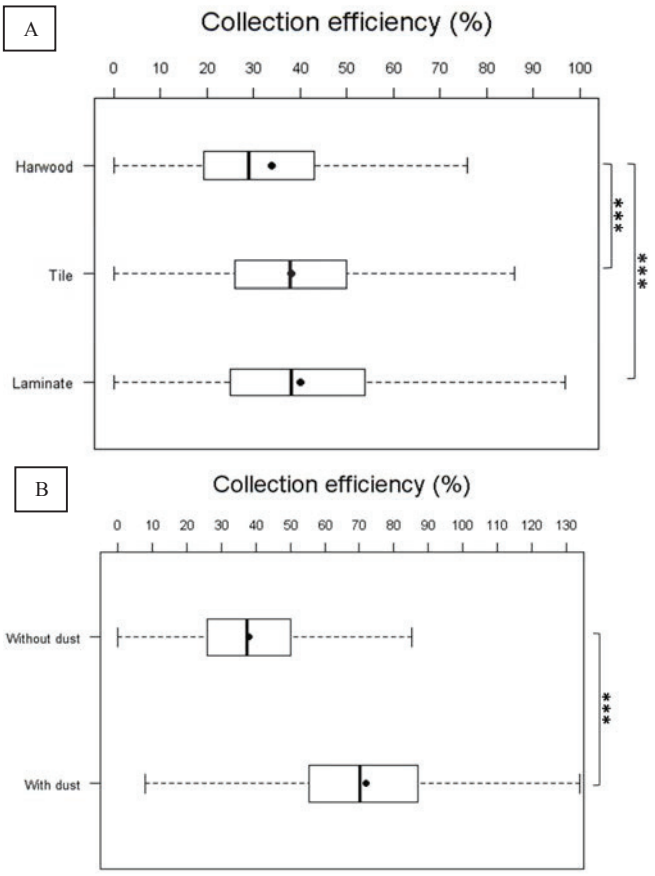
III.2.3 Results

a) Experiment one

Six of the 48 compounds analysed were below the quantification limits for at least one of the tested surfaces (dimetilan and imidacloprid for all test surfaces). The overall collection efficiency across all chemicals was 38% on tile, 40% on laminate, and 34% on hardwood (37, 39, and 34% for pesticides and 44, 45, and 32% for PCBs, respectively). No statistical differences were observed on overall collection efficiency between tile and laminate surfaces. By contrast, the overall collection efficiency on hardwood was significantly lower (tile vs. hardwood: $p < 0.001$; laminate vs. hardwood: $p < 0.001$) (Figure 3.1). The individual collection efficiencies were 70–110% for four of the pesticides (spiroxamine on tile and laminate, chlorpyrifos-methyl on laminate and hardwood, deltamethrin on tile and kresoxim-methyl on hardwood). The individual collection efficiencies were 50–70% for five of the pesticides (chlorpropham, chlorpyrifos-ethyl, chlorpyrifos-methyl, flufenoxuron, and permethrin) and two of the PCBs (PCB 101 and PCB 28) on tile; eight of the pesticides (chlorpropham, chlorpyrifos-ethyl, deltamethrin, diazinon, flufenoxuron, folpet, pendimethalin, and permethrin) and two of the PCBs (PCB 101 and PCB 28) on laminate; and eight of the pesticides on hardwood (chlorpropham, chorpyrifos-ethyl, cypermethrin, deltamethrin, dimethenamid-P, folpet, pendimethalin, and spiroxamine) (Table 3.1).

Significant differences in the collection efficiency were found between the surfaces for 16 of the pesticides (40%) and six of the PCBs (75%). Among these, differences were found between hardwood and tile for 10 pesticides and 6 PCBs (lower on hardwood, except for chlorpyriphos-methyl). Differences were also found between hardwood and laminate for 11 of the pesticides and four of the PCBs (lower on hardwood, except for dimethenamid-P). The collection efficiencies on laminate and tile were similar, except for pendimethalin (higher on laminate) (Table 3.1). The redundancy analysis and permutation test showed that the surface type accounted for 12% of the variability explained and had a significant impact on the collection efficiency ($p < 0.05$). The mean

repeatability was 55% on tile, 50% on laminate, and 62% on hardwood (61, 54 and 70% for pesticides and 29, 33 and 27% for PCBs, respectively). When considering only RSDs below 100%, the mean repeatability in experiment one was 37% for each surface. The repeatability was below 20% for three pesticides on laminate (dimethomorph, flufenoxuron, myclobutanil), and four pesticides and two PCBs on hardwood (acetochlor, fipronil, metolachlor, spiroxamine, PCB 118, PCB 138). A repeatability between 20 and 30% was observed for nine pesticides and five PCBs on tile, 10 pesticides and three PCBs on laminate and seven pesticides and four PCBs on hardwood (Table 3.1).

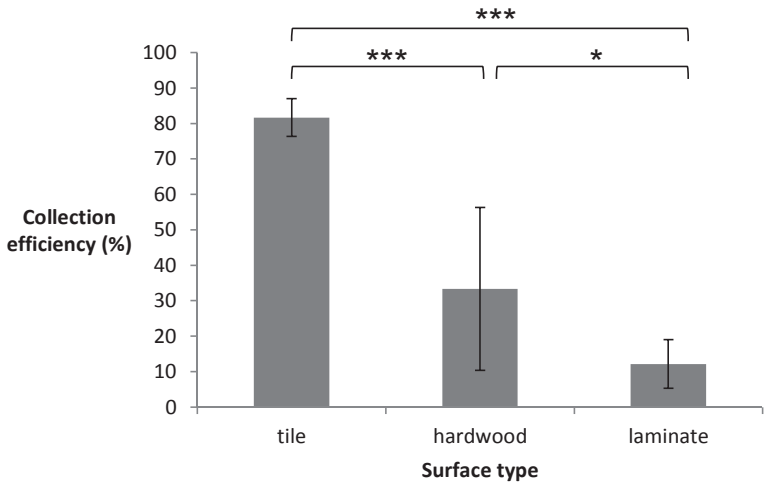


*Panel A shows the collection efficiency of the 48 compounds spiked on three test surfaces (experiment one), and panel B shows the collection efficiency of the 48 compounds in the presence and absence of dust (experiment three). Boxes indicate the interquartile range (IQR) with mean (dot) and median (line). Bars reflect most extreme point within 1.5 times the IQR. *** corresponds to p-value < 0.001.*

Figure 3.1: Wipe overall collection efficiency for the 48 Pesticides-PCBs

b) Experiment two

Figure 3.2 presents the efficiency and the repeatability of the wipe for dust collection on the three test surfaces. Sampling dust on tile showed the highest collection efficiency (mean 82%) and a good repeatability (6%). Both hardwood and laminate surfaces showed poor collection efficiency (mean 33% and 12%, respectively) and poor repeatability (69% and 56%, respectively). The results differed significantly between the three surfaces (tile vs. hardwood, $p < 0.001$; tile vs. laminate, $p < 0.001$; hardwood vs. laminate, $p < 0.05$).



Asterisks indicate the statistical differences between two groups: (*) p-value < 0.05; (***) p-value < 0.001.

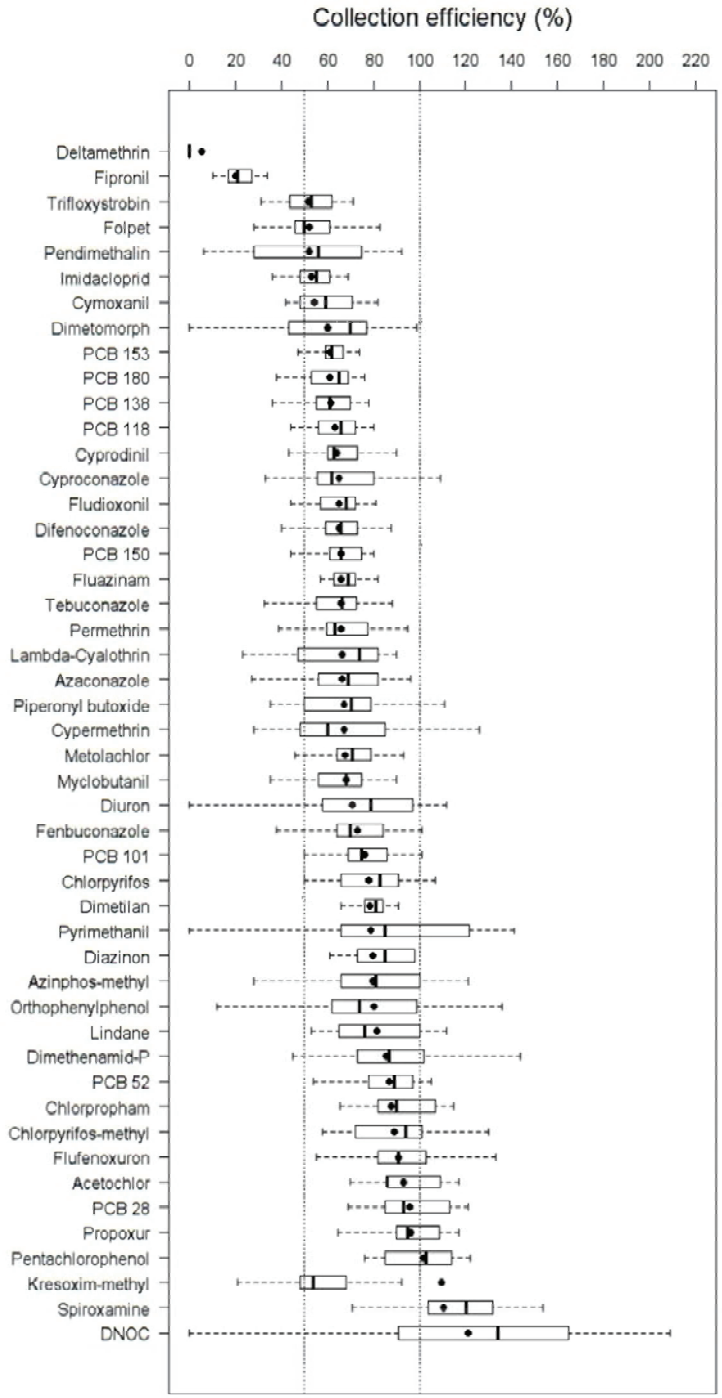
Figure 3.2: Mean dust collection efficiency and standard deviation (bars) for the 3 test surfaces (experiment two)

c) Experiment three

All of the 48 compounds tested were quantifiable when spiked on dust; however, dimetilan, dinitro-*ortho*-cresol (DNOC), and imidacloprid were not detected when they were directly spiked on tile (experiment one). The overall collection efficiency across all chemicals was 38% without dust and 72% with dust (37% and 72% for the pesticides and

44% and 71% for the PCBs, respectively). When the compounds were spiked onto the dust, the overall collection efficiency was significantly higher ($p < 0.001$) (Figure 3.3). Individually, we found collection efficiencies of 70–110% for 17 of the pesticides and three of the PCBs and 50–70% for 19 of the pesticides and five of the PCBs (Figure 3.3). The collection efficiencies were below 50% for deltamethrin and fipronil, and over 110% for DNOC and spiroxamine (Table 3.1).

By contrast, when compounds where directly spiked onto the tile, we observed 37 pesticides and two PCBs with collection efficiencies below 50% or over 110%. The collection efficiencies were significantly better in the presence of dust compared to the absence of dust for 38 of the 40 pesticides and all PCBs ($p < 0.001$ for 23 of the pesticides and two of the PCBs, $p < 0.01$ for six of the pesticides and two of the PCBs and $p < 0.05$ for nine of the pesticides and one of the PCB) (Table 3.1). For fipronil and deltamethrin, better collection efficiencies were observed in absence of dust ($p < 0.05$ and $p < 0.01$, respectively). According to the redundancy analysis and permutation test, dust (presence/absence) accounted for 50% of the data variability and had a significant impact on the collection efficiency ($p<0.01$). The mean repeatability was 55% in the absence of dust and 39% in the presence of dust (61% and 43% for the pesticides, and 29% and 18% for the PCBs, respectively). When considering only RSDs below 100%, the mean repeatability in experiment three was 37% in the absence of dust and 31% in the presence of dust. In the presence of dust, 18 of the pesticides and eight of the PCBs had a repeatability below 30% (including 11 compounds below 20%). In the absence of dust, 10 of the pesticides and five of the PCBs had a repeatability between 20 and 30% (none were below 20%).



The figure shows the individual collection efficiencies for the 48 compounds adsorbed on dust (experiment three). Boxes indicate the interquartile range (IQR) with mean (dot) and median (line). Bars represent values within 1.5 IQR.

Figure 3.3: Wipe collection efficiencies in presence of dust for 48 compounds (experiment three)

d) Physicochemical properties and collection efficiencies

Table 3.3 presents Spearman's correlation coefficient between the physicochemical properties and collection efficiencies of all of the compounds (each replicate considered) for experiments one and three. In experiment one, positive correlations were found between the collection efficiencies and log Koc (r = 0.3, 0.3, and 0.2 for tile, laminate, and hardwood, respectively; $p < 0.001$) and log Kow (r = 0.4, 0.4, and 0.3, $p < 0.001$). Negative correlations were found between the collection efficiencies and log Koa (r = -0.1 and -0.2 for tile and hardwood, respectively; $p < 0.001$) and the solubility in water (r = -0.3, -0.4, and -0.3 for tile, laminate, and hardwood, respectively; $p < 0.001$). In the presence of dust (experiment three), the correlation between the collection efficiencies and log Koc, log Kow, log Koa and solubility in water were inversed (r = -0.2, -0.1, 0.2 and 0.2, respectively; $p < 0.001$ for all).

Table 3.3: Correlation between collection efficiency and physicochemical properties

		Experiment one			Experiment three	
		Tile (n=17)	**Laminate (n=11)**	**Hardwood (n=11)**	**In absence of dust (n=17)**	**In presence of dust (n=17)**
Log Kow	**p-value**	<0.001	<0.001	<0.001	<0.001	<0.001
	r	0,4	0,4	0,3	0,4	-0,1
Log Koa	**p-value**	<0.001	NS	<0.001	<0.001	<0.001
	r	-0,1	-0,1	-0,2	-0,1	-0,2
Log Koc	**p-value**	<0.001	<0.001	<0.001	<0.001	<0.001
	r	0,3	0,3	0,2	0,3	-0,2
Solubility in water	**p-value**	<0.001	<0.001	<0.001	<0.001	<0.001
	r	-0,3	-0,4	-0,2	-0,3	0,2

NS: non-significant ($p \geq 0.05$); n: number of replicates per experimental condition. Coefficients of correlation (r) were determined by Spearman's correlation tests.

III.2.4 Discussion

To our knowledge, this is the first study to examine the efficiency and repeatability of wipe collection with a protocol that included up to 57 compounds (48 pesticides, eight PCBs, and one pesticide synergist). This study was also the first to test cellulose wipes. Three floor types were tested, corresponding to the floor types frequently found in French households. We used a mixture of pesticides and PCBs at environmental concentrations to mimic field situations. For the first time, synthetic dust was used to characterise the wipe accuracy and precision for pesticides and PCBs to be the closest with real situation of indoor dust sampling.

Although there were differences in their experimental study design, previous studies have investigated wipe collection efficiency by applying pesticides or other organic compounds directly to surfaces (Bernard et al., 2008; Deziel et al., 2011; Madireddy et al., 2013; Nussbaumer et al., 2012; Sottani et al., 2007; Willison, 2012). Our first experiment was set up based on these publications, in particular the studies of Bernard et al. (2008) and Deziel et al. (2011). However, in field conditions, pesticides and other organic compounds are often adsorbed onto organic particles, in particular dust, instead of directly deposited onto a sampling surface (Weschler and Nazaroff, 2008). Based on this observation, we designed experiment two and three.

The low concentrations used in our study may explain the lower accuracy and poorer precision we found compared to the existing literature. Deziel et al. (2011) showed that a lower concentration of pesticide solution was associated with lower collection efficiencies and that measurement variability was higher when concentrations were closer to the limit of detection. Our average concentration was 50× LQ, and the corresponding mass per area (0.03 ng/cm²) was at least 3,000 times lower than the one used by Bernard et al. (2008) and 600–1,800 times lower than the study from Rohrer et al. (2003). Our average concentration was similar to the low concentrations tested by Deziel et al. (2011) for 14 of their compounds but lower for the 13 other compounds (3–116 times lower). For four of the 14 compounds tested at similar loads, the individual collection efficiency

on tile was lower in our study compared to Deziel's study (diazinon: -4%, permethrin: -9%, fipronil: -13%, and piperonil butoxide: -15%). They suggested that the stainless steel surface used in their experiments might explain the higher collection efficiencies compared to those observed for surfaces usually found in households, such as those used in our study. In their study on the efficiency of wipe collection of chemical warfare agents, Willison (2012) attributed the lower recovery found on wood to its greater porosity compared to laminate, metal, glass, and vinyl tile. Rohrer et al. (2003) showed that pesticide collection efficiency using gauze wipes was generally better on tile compared to hardwood and that hard surfaces had better wipe collection efficiencies compared to carpet. Carr and Hill (1989) showed that higher surface porosity was associated with lower collection efficiency. However, Bernard et al. (2008) found no significant differences in the efficiency of pesticide collection between hardwood flooring and ceramic or vinyl tile. Interestingly, roughness seemed to have an impact on the efficiency and repeatability of dust collection in our study; these were significantly lower for the two rough surfaces (laminate and hardwood) compared to tile. Our results indicate that porosity and roughness may impact collection efficiency and that sampling surfaces should be chosen carefully.

Despite similar vapour pressures, Madireddy et al. (2013) observed strong variations in the collection efficiencies between heroin and cocaine. Based on these findings, the authors suggested that physical and/or chemical interactions, solvent polarity, and the volatility of compounds may affect collection efficiency. While the structures of pesticides and PCBs are different from drugs, they are both organic compounds and would share some physicochemical properties. The authors further suggested that important amounts of the compounds may not be recovered, even after two wiping, because of interactions between the surface and the compounds. In our study, the significant improvement in collection efficiencies that we observed in the presence of dust may be explained by a modification of the physicochemical interactions of the compounds with the surface, the wipe and/or the solvent. The physicochemical properties assessed were selected to express different compound characteristics: low log Kow and high solubility in water characterise more hydrophilic compounds; high log Koc

characterises compounds with high affinity for organic carbons (including soil and dust particles). Analogously, high log Koa values reflect high affinity for particles compared to air. Considering the affinity of the extraction solvent (dichloromethane) for apolar compounds, we expected good collection efficiencies for compounds having high log Kow and a low solubility in water. This was observed in the absence of dust in our analyses and has been suggested in previous studies (Deziel et al., 2011; Madireddy et al., 2013; Mercier et al., 2011). It could explain why more hydrophilic compounds (low log Kow and high solubility in water) were not quantified in the positive controls in experiments one and three (dicamba, mesotrione, sulcotrione, and iodocarb only in experiment one). We also expected that the presence of dust would favour the collection efficiency of compounds with a higher Koc (higher affinity for dust and particles). Interestingly, we observed the opposite, probably because some of these compounds have higher affinity for the dust compared to the extraction solvent. Furthermore, the direction of the correlation of the collection efficiency with Kow and solubility in water changed in the presence of dust (despite increased collection efficiency for more lipophilic compounds). This was not surprising since Koc and Kow are correlated (Baker et al, 2000). Also, we hypothesised that a lower Koa would be associated to an easier desorption from particles and wipes but we observed a negative correlation for all experiments. Overall, our results suggest that dust influences physicochemical interactions between compounds, wipes, and the extraction solvent. However, it seems difficult to predict wipe performance based on these characteristics alone, partly due to the low correlation coefficients observed. For example, similar collection efficiencies were found for pesticides and PCBs, despite PCBs having a higher log Kow. Moreover, dust composition and properties, which may vary between homes, may influence physicochemical interactions.

To our knowledge, the standard reference material (SRM) 2585 produced by the US *National Institute of Standards and Technology* is the only indoor dust certified as a reference for analysing organic contaminants (Poster et al., 2007). This dust has the advantage of providing a material that is close to field conditions. However, indoor dust composition varies between households (Mercier et al., 2011) and SRM 2585 was

developed based on a blend of US samples from the 1990's, which may be different from the current housedust in France. Standard dust has a standardised particle size, which would provide more homogenous adsorption and distribution on a sampling surface and has the advantage of almost no contamination compared to SRM 2585, which contains at least 140 known organic compounds (Poster et al., 2007). According to a previous Danish study (Molhave et al. 2000), ASHRAE synthetic dust appeared to have similar properties in terms of particle size than SDRs. The composition varied slightly but remained on a same order of magnitude (ASHRAE: 23% organic matter, 8% inorganic matter, and 5% fibres; Danish housedust: 33% organic matter, 12% inorganic matter, and 0.2% fibres).

The results from experiment two showed that cellulose wipes can collect 82% of the synthetic dust on tile with a good repeatability. This indicates that collection of synthetic dust itself should had only a small impact on repeatability in experiment three, facilitating the interpretation of the wipe performance in this experiment. These findings also suggest that loss of dust during the transfer from the aluminium foil to the sampling surface, as well as potential particle clumping, had minor impact in our experiment. Although we tried to approach a real housedust sampling condition, our experiment may not completely fit this ideal, especially for the elapsed time between the adsorption of the organic compound to the dust and the wipe sampling. However, compounds have been shown to be stable in dust, where they are protected from environmental degradation, and can persist for long periods (Lewis et al., 1999).

The individual collection efficiencies in the presence of dust were 50 – 110% for all of the compounds, except fipronil and deltamethrin. A low collection efficiency for fipronil was previously described by Bernard et al. (2008) and Deziel et al. (2011) and may occur from losses during extraction, particularly at low concentrations. Bernard et al. (2008) found that deltamethrin had the lowest collection efficiency compared to the other pyrethroids tested. In our study, in the absence of dust, the individual collection efficiency of deltamethrin was 100% on average on tile, and above the collection efficiencies of other pyrethroids (permethrin, cypermethrin, and λ-cyhalothrin). However, this did not occur in the presence of dust (collection efficiency: 5%). This could be

explained by the low water solubility (0.2 µg/L) and the high log Koc (7.01) of deltamethrin, which could confer a high affinity for the organic carbon particles present in dust. Indeed, this affinity may have been higher than that for dichloromethane; therefore, deltamethrin may have remained adsorbed on the dust during the extraction.

Some sampling materials have been shown to contain interfering compounds (Stout II et al., 2009), highlighting the importance to control this medium. In this study, we tested the wipes before cleaning and we did not found any pesticides or PCBs on them. However, background noise and traces of polycyclic aromatic hydrocarbons and mineral oils were detected, which could impact our ability to interpret our results. Moreover, the wipe boxes provided by the supplier were not perfectly sealed, and sporadic contamination cannot be ruled out. Therefore, we decided to systematically pre-clean our cellulose wipes using dichloromethane (99.9% purity).

Because some pesticide and PCB residues may have persisted on the surfaces after wiping, we did not subtract the negative control values from the sample concentration to determine the collection efficiency. However, no pesticides or PCBs were quantified (< LQ) in 66% of the negative controls, and pesticides and PCBs that were detected in the negative controls were below the sample concentrations (except for two compounds that were excluded from the statistical analysis). Interestingly, we detected less pesticides in the negative controls performed on tile compared to the laminate and hardwood surfaces (experiment one).

Our study presents several limitations. Despite thorough cleaning of the surfaces between each replicate, persistence of compounds on the sampling surface between replicates might have influenced the collection efficiency and decreased the repeatability. However, no clear augmentation of the collection efficiency or increasing contamination of the negative control was observed. We observed poor repeatability for the pesticides tested, which comprised various chemical families, compared to the PCBs, which corresponded to a homogenous chemical group. By considering one chemical family at a time, the repeatability should improve. Also, we cannot guaranty the complete homogenous

adsorption of pesticides on the synthetic dust or the complete homogenous distribution of the dust on the sampling surface (experiment three). In a future study, it would be pertinent to assess different methods of adsorbing organic compounds on dust and their impact on the collection efficiency. Additionally, part of the pesticide solution spiked on the synthetic dust may have been lost on the aluminium foil instead of adsorbed. However, these limitations would have increased the variability between the replicates and positive controls and reduced our ability to show significant impact of physicochemical properties, but not increased the overall collection efficiency, compared to the one obtained in absence of dust. Because we observed better repeatability in experiment three compared to experiment one (without dust), and since the standard deviations of the positive controls remained low, the impact of these on our results was probably minor. Another potential limitation is related to our sampling procedure. Because we only used wipes saturated with isopropanol for sampling, pesticides might have remained on the sampling surface with the solvent. This may have been avoided by using a dry wipe after the wetted wipe, as performed by Bernard et al. (2008) and Vonderheide et al. (2009). Also, there could have been a potential loss of pesticides and PCBs during the evaporation of isopropanol (10 h). However, because we estimated the collection efficiency by comparing the replicates to the positive controls, this phenomenon should only have a limited impact (the replicates and positive controls were made using the same procedures). Finally, the low quantity of dust sampled using wipes (compared to other methods, e.g. vacuum cleaner) may have reduced our ability to detect some compounds, which remains an important barrier to its routine use (Mercier et al., 2011).

In this study, the presence of dust significantly improved the collection efficiency of the cellulose wipes. Therefore, a too low quantity of dust may lead to underestimate pesticide and PCB levels in households; however, the optimal dust quantity to ensure the best efficiency of wipes as well as the optimal size of the sampling area remains to be determined. The sampling area should be large enough to get a sufficient quantity of dust. In the literature, wiped areas varied from 0.03 to 1.2 m² (Mercier et al. 2011). Based on our findings, pre-cleaning of the sampling surface and a standardised duration of dust

deposition prior to sampling would improve the comparability of the concentration of compounds detected.

III.2.5 Conclusion

Indoor dust samplings are increasingly used in epidemiological studies to characterise environmental exposure to pesticides and PCBs. Surface wiping is an easy and efficient method to collect indoor dust for large-scale studies, but the varying study designs underpin the need for further validation and standardisation. In this context, our study presents a promising approach to test the efficiency of wipe collection for pesticide and PCB residues in indoor dust. The conditions in our experiments, in particular the large number of compounds studied at environmental concentrations and the choice of test surfaces, mimicked field conditions. The use of synthetic dust in our experiment was also innovative and should be considered in future validation studies. We found a good overall collection efficiency for a large number of pesticides and PCBs when adsorbed on dust, indicating that our cellulose wipes might be relevant for sampling organic compounds in indoor dust. Hard and smooth surfaces, such as stoneware tile seem to be the most appropriate sampling surfaces with our method. Our results contribute to a better understanding about the impact of factors such as surface characteristics and physicochemical properties on wipe sampling, providing useful information to researchers for study design and analyses of field results obtained by this method.

Acknowledgements:
The authors would like to acknowledge the Departmental laboratory of the Drôme Department, France, for their support during the experimentations, and Sophie Domingues (KOONEC) for the editorial assistance. Rémi Béranger holds a doctoral grant from the *Région Rhône-Alpes*. This project was funded by the *Fondation de France* (Engt 2011-00023939).

III.2.6 Supplemental Materials

Efficiency of wipe sampling on hard surfaces for pesticides and PCBs residues in dust

Joane Cettier, Marie-Laure Bayle, Rémi Béranger, Elise Billoir, John R. Nuckols, Bruno Combourieu, Béatrice Fervers

Table S1. Concentrations of negative controls

	Spiking solution S1[a] (µg/L)	Negative control on laminate[b], without dust: mean µg/L (SD)	Negative control on hardwood[b], without dust: mean µg/L (SD)	Negative control on tile[b], without dust: mean µg/L (SD)	Negative control on tile[b], with dust: mean µg/L (SD)
Acetochlor	79	1 (1)	1 (1)	0	0
Azaconazole	56	0	0	0	0
Azinphos methyl	45	0	0	0	0
Chlorpropham	54	1 (1)	1 (1)	0	0
Chlorpyriphos ethyl	48	2 (2)	1 (1)	0	0
Chlorpyriphos Methyl	29	2 (2)	1 (1)	0	0
Cymoxanil	47	0	0	0	0
Cypermethrin	48	0	0	0	0
Cyproconazole	44	0	0	0	0
Cyprodinil	48	2 (2)	2 (2)	0	0
Deltamethrin	46	0	0	0	0
Diazinon	13	0	0	0	0
Difenoconazole	43	0	0	0	0
Dimethenamide-P	36	2 (1)	1 (1)	0	0
Dimetilan	53	0	0	0	0
Dimethomorph	61	7 (10)	0	0	0
Diuron	43	0	0	0	0
DNOC	98	0	0	0	0
Fenbuconazole	51	0	0	0	0
Fipronil	51	3 (2)	2 (2)	0	0
Fluazinam	95	0	0	0	0
Fludioxynil	35	4 (5)	2(3)	0	0
Flufenoxuron	101	5 (6)	6 (8)	0	0
Folpet	33	9 (13)	0	0	0
Imidacloprid	52	0	0	0	0
Kresoxim methyl	66	0	0	0	0

λ-Cyalothrin	60	6 (4)	5 (6)	5 (1)	0
Lindane	54	0	0	0	0
Metolachlor	39	3 (4)	4 (1)	1 (1)	0
Myclobutanil	50	0	0	0	0
Orthophenylphenol	42	5 (3)	4 (1)	2 (1)	0
PCB 101	61	6 (2)	2 (3)	2 (1)	0
PCB 105	71	8 (4)	4 (5)	3 (1)	0
PCB 118	68	8 (6)	2 (3)	1 (1)	0
PCB 138	80	8 (4)	3 (4)	2 (3)	0
PCB 153	74	6 (3)	3 (4)	3 (0)	0
PCB 180	69	7 (5)	4 (2)	3 (1)	0
PCB 28	33	3 (2)	1 (1)	1 (0)	0
PCB 52	42	5 (6)	1 (1)	0	0
Pendimethalin	58	0	0	0	0
Pentachlorophenol	120	0	0	0	0
Permethrin	45	7 (9)	6 (8)	0	0
Piperonyl Butoxide	48	3 (4)	2 (3)	0	0
Propoxur	25	1 (1)	1 (1)	0	0
Pyrimethanil	25	1 (1)	0	0	0
Spiroxamine	90	0	5 (7)	0	0
Tebuconazole	58	0	0	0	0
Trifloxystrobin	55	5 (6)	3 (4)	0	0

SD: standard deviation.
[a]:Concentrations of compounds in the spiking solution are based on 3 repeated analyses.
[b]:Two negative controls were performed for each experimental condition.

Table S2. Analytical conditions for HPLC/MS-MS and GC/MS

	HPLC	GC
Injection volume	10 µL	5 µL
Mobile phase	water : acetonitrile (1 : 1) acidified by 0.1% formic acid	helium
Flow	250 µL/min	1 mL/min
Stationary phase	Grace Alltima non polar C18 column (Grace, Deerfield, USA)	GC DB-5 non polar column (Agilent Technologies, Santa Clara, USA)
Column	Length: 150 mm Internal diameter : 2.1 mm Particle size: 5 µm	Length: 30 m Internal diameter : 0.25 mm Thickness: 0.25 µm
Temperature	20°C	50°C to 140°C (20°C/min) 140°C to 320°C (8°C/min) 11 min at 320°C
	MS-MS	**MS**
Ionization source	Electrospray (ESI)	Electron impact
Detector	Triple quadrupole, MRM mode	Ion trap

HPLC: High performance liquid chromatography; GC: gas chromatography; MS: mass spectrometry; ESI: electrospray ionisation; MRM: multiple reaction monitoring

Figure S1. Photographs of the three sampling surfaces

From the left to the right: laminate, hardwood, tile.

III.3 (article #3)

Agricultural and domestic pesticides in house dust from different agricultural areas in France

Rémi Béranger[1,2,3], Elise Billoir[4], John R Nuckols[5,6], Jeffrey Blain[1], Bruno Combourieu[4], Therry Philip[1], Joachim Schüz[2]*, Béatrice Fervers[1,3]*

(1) Unité Cancer et Environnement, Centre Léon Bérard, Lyon, France

(2) Section of Environment and Radiation, International Agency for Research on Cancer (IARC), Lyon, France

(3) EAM 4128, Université Claude Bernard, Lyon, France

(4) Rovaltain Research Facility for Environmental Toxicology and Ecotoxicology, Valence, France

(5) Dept. of Environ and Radiol. Health Sci, Colorado State University, Fort Collins, CO, USA

(6) Principal, JRN Environmental Health Sciences, Ltd, North Bethesda, MD, USA

*The last two authors contributed equally to this work and share last authorship

Article submitted to *Environmental Health Perspective*

III.3.1 Introduction

Exposure to pesticides have been suggested as a risk factor for several diseases or adverse outcome, such as cancer (Alavanja and Bonner 2012), Parkinson's disease (Noyce et al. 2012), birth defects (Damgaard et al. 2006), and infertility (Bretveld et al. 2007). There is growing evidence of pesticide contamination of indoor residential environments, either from domestic use or transport and deposition from outdoor sources, e.g. applications on nearby agricultural fields (Mercier et al. 2011). People spend about 85 to 90% of their time indoor, largely at home, where chemicals in the dust can be ingested, inhaled, or absorbed through the skin (Butte and Heinzow 2002). House dust is a repository of various chemicals and house dust sampling is an efficient method to measure household pesticide contamination (Lioy et al. 2002;Colt et al. 2004). Because of protection from degradation by sunlight, fungus, and other factors, pesticides in indoor dust are more stable over time than outdoor (Butte and Heinzow 2002).

Using hard surface floor wipes, the American Health Home Survey screened 24 compounds including agricultural, domestic and banned insecticides in 500 nationwide US households (Stout et al. 2009). Frequency of detection in house dust varied from 0.4 to 89% depending on the compounds. Most households had detectable levels of insecticides. Using carpet vacuum samples, Quiros-Alcala et al. (2011) reported higher prevalence of pesticides in 15 farmworker homes compared to 13 urban, non-farmworker homes. However, among the 22 pesticides screened, six were detected in at least 79% of all homes. Previous studies have detected domestic pesticides in house dust (Mercier et al. 2011), and Colt et al. (2004) showed correlations between self-reported domestic pesticides use and detection of 15 pesticides in vacuum bag samples from 513 Californian households. However, only a fraction of the variability of domestic pesticide concentrations was explained by self-reported use of domestic pesticides (r^2= 0.09 – 0.39).

Studies of pesticides in house dust have mainly relied on vacuum or wipes dust sampling (Mercier et al. 2011). Wipes were preferred in large-scale studies for its ease of use

(Deziel et al. 2011; Mercier et al. 2011). However, most available studies focused on a limited number of pesticides or households and only few surveys were conducted outside the US. Our study aimed to characterize pesticide contamination of homes proximate to typical crops grown in the Rhône-Alpes region (France), including the relative occurrence of agricultural and non-agricultural pesticides, using house dust wipe sampling.

III.3.2 Methods

a) Study population

Our study was conducted in four areas within the Rhône-Alpes region, France. We chose three zones representing major agricultural practices in the region: orchards and cereals (Zone 1), cereals only – mainly corn and grain (Zone 2), and vineyards (Zone 3). An urban area with a "zero-pesticide-use" policy since 2008 was chosen as control area (Zone 4).

Using a Geographic Information System (GIS), we selected households located less than 1000 meters from peach and apricot orchards (Zone 1), corn and grain crop fields (Zone 2), and vineyards (Zone 3). Control households (Zone 4) were selected at least 2000 meters away from agricultural fields and 500 meters away from railway, highway, or major public parks. Location and crop type were identified through the 2006 CORINE Land Cover® database (http://sd1878-2.sivit.org/) and data provided by the Departmental Agricultural Chambers (DAC).

Out of 645 eligible households contacted by telephone or volunteering in Zones 1-3, 442 declined participation or were excluded due to occupational pesticide use of household members, leaving 203 households included (69 in Zone 1, 66 in Zone 2, 68 in Zone 3). Additionally, 36 households without occupational pesticide users were recruited in Zone 4 through email contacts. All participants signed consent forms. The study was approved

by relevant French authorities (French National Commission of Informatics and Freedom, CNIL – n°1560501v0).

b) Data collection

Households were visited twice in 2012, spaced 30 days apart, during the predominant period of agricultural pesticide use on targeted crops (Zone 1: April-May; Zone 2: April-June; Zone 3: June-July) according to the DAC and previous air-quality measurements (ATMO Drôme-Ardeche 2010). Zone 4 was sampled during periods of low agricultural pesticide use (October-November).

During the first visit, a trained investigator (RB or JB) collected consent forms, measured geographical coordinates using a Tomtom® XL GPS receiver (TomTom NV, The Netherland), and administered a standardized questionnaire concerning household characteristics (number of inhabitants; floor level; presence of pets; domestic pesticide use for pets, outdoor gardens, indoor plants, insects, and woodwork/framework during the two previous years). Investigators also asked to see pesticide packaging to identify active ingredients. Whenever possible, missing responses were completed during the second visit.

Using ArcGIS software 9.3 (ESRI, Redlands, CA), we mapped the location of households from their GPS coordinates, and determined area and crop type within 1000m using 2012 spatially registered land cover data for vineyards ("Casier Viticole Automatisé", General directorate of customs and excise (DGDDI)) and other crop types ("Registre Parcellaire Graphique", Regional directorate for food, agriculture and forestry (DRAAF)). Crop locations and types were field-verified (RB and JB).

c) Dust sample devices and methods

In each home, we collected two "recent dust" samples (RDS), one passive (dust trap) and one active (floor wipe); and one "old dust" sample (ODS). The dust trap was similar to one reported by Edwards et al. (1998), and composed of a 22.8cm x 22.8cm non-electrostatic pure polypropylene wipe (Kimtech pure W4 ref. 7646, Kimberly-Clark® professional, UK) fixed on an untreated wood frame using iron pins. During the first visit, the dust trap was placed near a main entrance door (maximum three meters) at one meter high or, when impossible, on the floor or on hard surface furniture, and left in place for approximately 30 days. The wipe was removed from the trap by gloved hands at the second visit.

The "floor wipe" sampling area (1 m^2) was defined during the first visit. The homeowner was asked not to clean this area for seven consecutive days prior to sampling (second visit). The sampling area was close to a main entrance door (maximum two meters), at least 10 cm from a wall or door. When impossible, we selected a cleared area in the kitchen or living-room. At the beginning of each sampling period cellulose wipes (Kimtech science ref. 7552, 11cm x 21cm, Kimberly-Clark® professional, UK) were purified using dichloromethane (99.9% purity, Carlo Erba Reagents, Milano, Italy) in a separating funnel (250 ml for 20 wipes), agitated one minute, and stored in similarly decontaminated glass boxes for transport. Two wipes moistened with isopropanol (10ml) were used per sample, as described by Cettier et al. (2014).

We employed essentially the same method for collecting ODS. During the first visit, we asked the owner to identify and to not clean an upper ledge of door or window frame in the living room, entranceway or kitchen where dust had accumulated for at least 6 months. During the second visit, we used 2 wipes to remove all dust from the sill.

All samples were placed in separate, clean, decontaminated, stoppered Pyrex flasks, stored in a cooler at ambient temperature immediately after sampling (icetime® 26 liters, Campingaz, France). Samples were transported by car within 3 days to the laboratory for

analysis. At the end of each sampling period, 2 cellulose wipes remaining in the glass storage container used for transport were moistened with isopropanol (10ml), placed in a flask, and transported to the lab to serve as blanks for quality control of the sampling procedure. The same procedure (without isopropanol) was followed for polypropylene wipes (dust trap). No contamination was found in blanks, except orthophenyphenol in two dust trap blanks performed in Zone 3, at concentrations similar to median.

d) Chemical analyses

We employed a method similar to that described by Bernard et al. (2008) to extract and measure the mass of 417 compounds in all samples (406 organic pesticides, 10 pesticide metabolites, and piperonyl butoxide (PB); Supplemental Materials, Table S1).

Laboratory methods are detailed in Supplemental Materials. Briefly, we extracted compounds by adding 150 mL of dichloromethane in each flask, which was agitated 4 hours. The solution was filtrated and concentrated to 1mL under nitrogen steam. The solution was separated in two equal parts and conditioned to be analyzed by gas chromatography coupled with mass spectrometer and by high performance liquid chromatography coupled with tandem of mass spectrometers. For all samples, extractions of internal standards (Chrysene D12, hexabromobenzene, and triphenylphosphate) were within 20 % the expected concentrations. The limit of detection by both methods was 1ng/mL for all compounds. Analyses were performed in accordance with international quality standards (ISO-17025, http://www.iso.org/).

e) Validation of sampling methods

We assessed repeatability and efficiency of the cellulose wipes by wiping synthetic dust spiked with a mixture of pesticides and PCBs at environmental concentrations (3.10^{-2}ng/cm²), using the same laboratory procedures. Details are described elsewhere (Cettier

et al. 2014). Based on 40 pesticides and eight PCBs, averaged wipe efficiency was 72% and averaged repeatability was 34%.

f) Categorization of pesticides

Pesticides authorized for agriculture or gardening in 2012 were listed in the registry of the French Agricultural Ministry (http://e-phy.agriculture.gouv.fr/) and the European Union Pesticide database (http://ec.europa.eu/sanco_pesticides/public/index.cfm). Pesticides authorized for gardening and/or reported to have domestic usage by study participants were categorized as domestic. Pesticides having both agricultural and domestic purposes were categorized as "mixed usage". The term "banned" was used to define pesticides not authorized for agriculture or gardening, and without reported domestic use.

We identified 29 agricultural pesticides most likely used on targeted crops during our sampling periods (Supplemental Material, Table S2) through data from the DAC of each study zone, farmers' pesticides use registries (Zone 1, Zone 3) and pesticide vendors (Zone 2).

g) Data analysis

We defined occurrence in RDS as the number of distinct compounds detected in each home, either from home's dust trap or floor wipe samples. For each zone, we defined the proportion of each class of pesticide (banned, agricultural, domestic, and mixed-use) across all households, in terms of overall occurrence in RDS and detection (presence/absence) in ODS. We calculated median and interquartile range of surface loading (SL) for each compound detected in dust trap and floor wipe samples separately. Due to limitations in measuring or standardizing the sampling area on window and door ledges, SL was not calculated for ODS.

We used Pearson's chi-squared test to compare frequency distribution between dust trap and wipe samples, RDS and ODS as well as across zones. The significance level was 5% ($p<0.05$). We performed multivariate analyses to investigate trends of overall agricultural pesticide contamination in households of the different zones, using Principal Coordinate Analysis (PCoA). PCoA is an unconstrained ordination method similar to Principal Component Analysis but adapted to a non-Euclidean simple presence/absence matching coefficient of dissimilarity (Borcard et al. 2011). We restricted this multivariate analysis to agricultural zones (1, 2, and 3) and agricultural pesticides, for RDS and ODS separately. Only households with detection of at least one agricultural pesticide were considered in our PCoA. Statistics were performed with R 3.0.0, using the ade4 (Dray and Dufour 2007) and vegan packages (Oksanen et al. 2012).

III.3.3 Results

a) Household characteristics and sources of exposures

Table 3.4 presents characteristics and reported domestic pesticide use of the 239 study households. Averaged number of household members was similar across zones (2.5 – 3). Households were mainly single level dwelling (89%). Eighty-five percent had a garden and 55% had a pet. Domestic pesticide use was reported by 209 households (87%): 179 (75%) to control indoor flying or crawling bugs, fungus and xylophage insects; 114 (48%) for gardening (56% of those having a garden); 88 (37%) to treat their pet(s) (67% of those having a pet).

In Zone 1, 21.6% of the surface within 1000m of study households (buffer) was covered by cereals (median values), 4.3% by peach and apricot orchards, and 0.1% by vineyards. In Zone 2, cereals represented 21.5% of the 1000m buffer surface. In Zone 3, 29.7% of the 1000m buffer surface was covered by vineyards and 2.5% by cereals. Other crops

types found within 1000m are detailed in Supplemental Materials, Table S3. In Zone 4, no crops were present within 1000m of households.

Table 3.4: Household characteristics and sources of exposures

	Zone 1 (n=69)	***Zone 2 (n=66)***	***Zone 3 (n=68)***	***Zone 4 (n=36)***
Inhabitant per households (median, IQR[a])	2 (2 – 4)	3 (2 – 4)	2 (2 – 4)	2 (2 – 4)
Floor level				
- Ground level: n (%)	67 (97%)	66 (100%)	67 (99%)	12 (33%)
Pets: n (%)	44 (64%)	38 (58%)	42 (62%)	8 (22%)
- Pesticides usage for pets: n (%)	*26 (59%)*	*27 (71%)*	*31 (74%)*	*4 (50%)*
Garden: n (%)	64 (93%)	55 (83%)	64 (94%)	20 (56%)
- Pesticides usage for gardening: n (%)	*29 (45%)*	*33 (60%)*	*43 (67%)*	*9 (45%)[d]*
Indoor uses of pesticides: n (%)	54 (78%)	53 (80%)	50 (74%)	22 (61%)[c]
Any domestic use of pesticides[b]: n (%)	62 (90%)	58 (88%)	66 (97%)	23 (64%)[c]
Proportion of agricultural fields within 1000m[d] (median, IQR[a])	46.8% (35 – 62.7)	39.5% (28 – 9.5)	53.2% (42.7 – 65.6)	none
- Orchards (peaches and apricots): median (IQR)	*4.3% (2.0 – 10.5)*	*none*	*none*	*none*
- Cereals (median, IQR)	*21.6% (12.9 – 30.2)*	*21.5% (14.5 – 31.8)*	*2.5% (0 – 9.8)*	*none*
- Vineyards (median, IQR)	*0.1% (0 – 1.4)*	*none*	*29.7% (6.3 – 48.3)*	*none*

[a]: *Inter-quartile range*
[b]: *Either pets, garden, plants, or indoor treatment (flying, crawling or xylophage bugs; fungus)*
[c]: *One missing value*
[d]: *Other crops type found are detailed in Supplemental Materials, Tables S3*

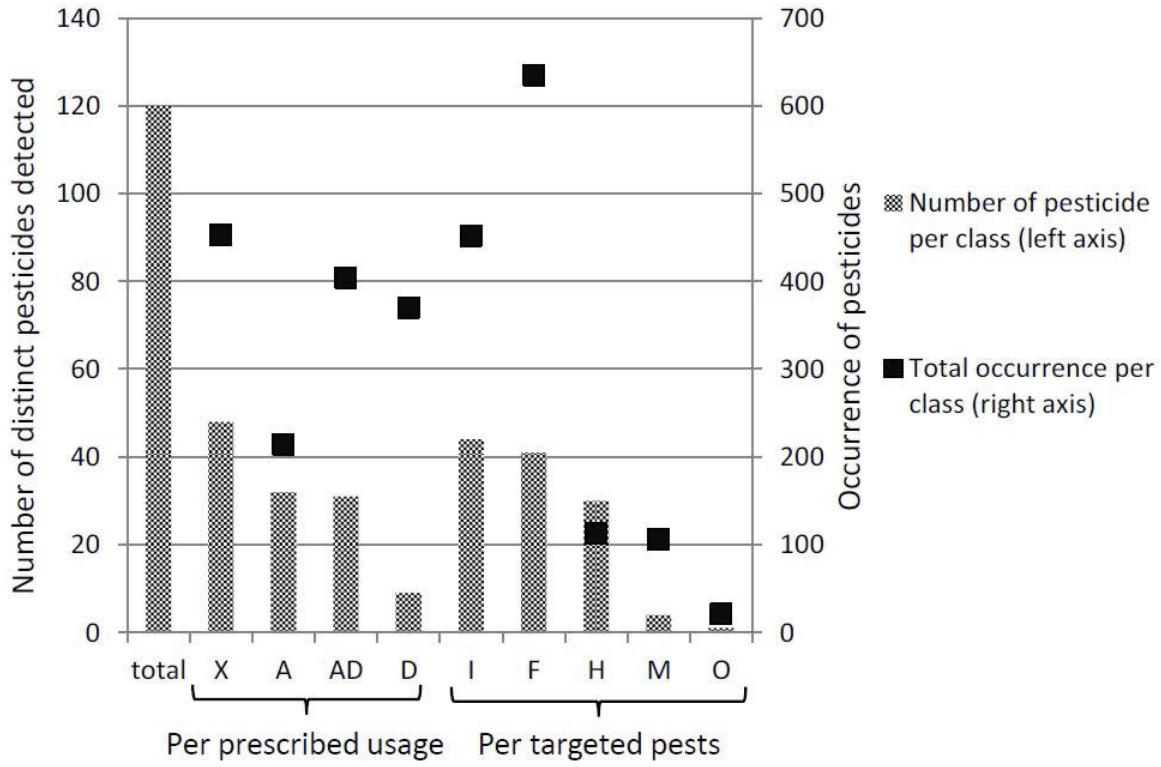
b) Compounds retrieved in recent dust samples

Overall, 125 different compounds were detected at least once in RDS (Figure 3.4): 120 pesticides (44 insecticides, 41 fungicides, 30 herbicides, 4 compounds targeting multiple pests, and anthraquinone, a bird repellent), PB and 4 DDT (dichlorodiphenyltrichloroethane) metabolites. Thirty-two pesticides were authorized for

agricultural use only, nine for domestic use only, 31 for mixed usage (agricultural and domestic) and 48 pesticides were considered as "banned" at sampling.

Summing the detections of the 120 pesticides across all study households resulted in a total of 1,327 occurrences (910 and 908 occurrences respectively in dust trap and floor wipe samples, Figure 3.4). Fungicides were most frequently detected (635 occurrences; 47%), followed by insecticides (452; 34%), herbicides (113; 9%), pesticides targeting multiple pests (106; 8%), and anthraquinone (21; 2%). Banned pesticides were most frequent (421occurrences; 32%), followed by domestic pesticides (370; 28%), pesticides with mixed usage (322; 24%), and agricultural pesticides (214; 16%). PB and DDT metabolites represented respectively 83 (6.2%) and 32 (2.4%) occurrences. Table S1 details the occurrence of individual compounds per zone.

Pesticide profiles in RDS varied between dust trap and floor wipe samples ($p<0.001$), as well as between zones ($p=0.4$). In Zone 1, a similar number of insecticides (n=27), herbicides (21), and fungicides (20) was detected. In Zone 2, insecticides (n=20) were more frequent than fungicides (12), and herbicides (8) ($p=0.4$). In Zone 3, fungicides (n=32) and insecticides (30) were more frequent than herbicides (13) ($p=0.2$). In Zone 4, frequencies were similar (10 insecticides, 7 fungicides, and 5 herbicides). Respectively 9, 8, 21, and 8 compounds were present in more than 15% of study households from Zones 1 to 4.



Prescribed usage: X (banned); A (agricultural use only); D (domestic use only); AD (both A and D). Targeted pests: I (insecticides); F (fungicides); H (herbicides); M (multiple targeted pests); O (other). Bars represent the number of pesticides detected at least once in recent dust (left y-axis). Dots indicate the pesticide occurrences summed across all study homes in recent dust (right y-axis). For recent dust, we considered one occurrence when pesticides were detected either in floor wipes or dust trap.

Figure 3.4: Profile of pesticides detected in recent dust in terms of usage and targeted pests, all zones combined

Overall, the ten most frequent compounds were orthophenylphenol, pentachlorophenol, piperonil butoxide, lindane, iodocarb, fipronil, tebuconazole, propiconazole, permethrin, cymoxanil (detection rate: 14% – 70%). Table 3.5 presents SL (ng/m^2) in dust trap and floor wipe samples for the 10 most frequent pesticides in each zone. Among these, domestic and banned compounds were predominant, except for Zone 3. Highest median SLs were observed for folpet (Zone 3), permethrin (Zone 2), lindane, orthophenylphenol, and PB (all zones).

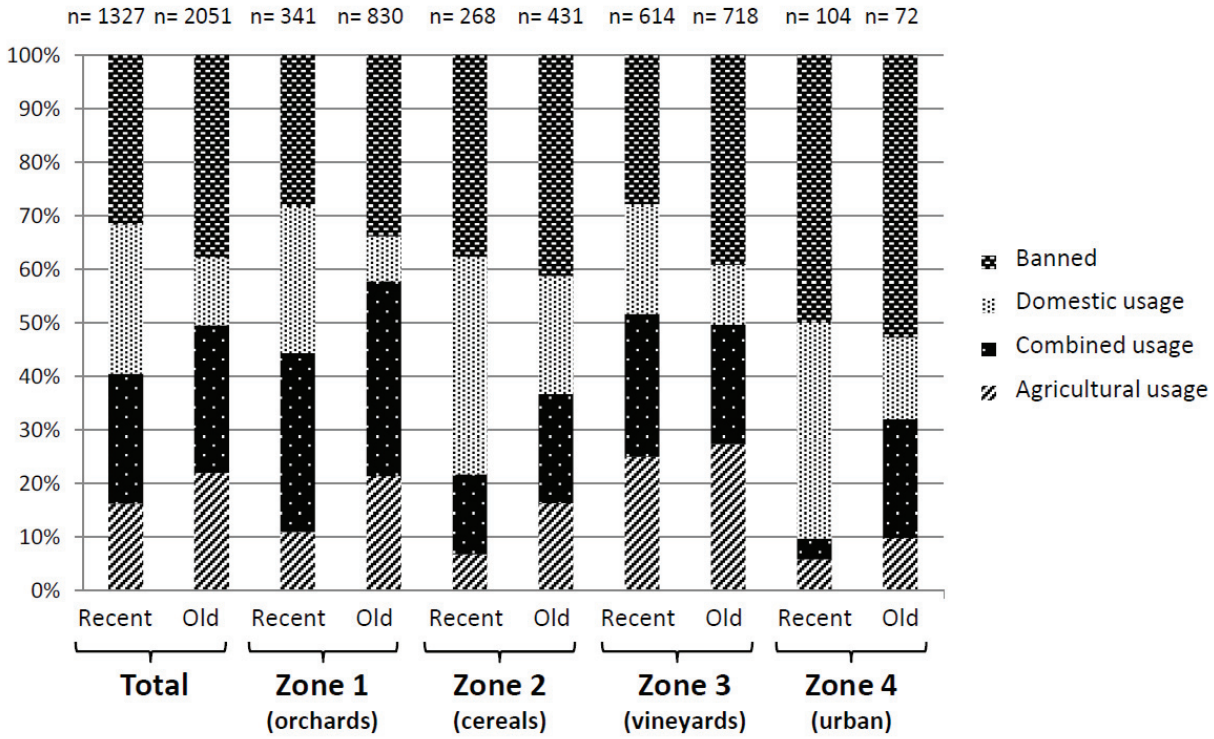
Table 3.5: Surface loading of the 10 most frequent compounds in recent dust samples for each zone

Name / zone	Status[a]	Occ.[b]	Dust trap (ng/m²) – 30 days			Floor wipes (ng/m²) – 7 days		
			ND[c]	Median	(Q25 – Q75)	ND[c]	Median	(Q25 – Q75)
ZONE 1 (69 households)								
1. Orthophenylphenol[d]	D	34	27	1,962	1,193 – 2,809	19	17	8 – 52
2. Pentachlorophenol	X	26	20	223	152 – 522	21	17	10 – 25
3. Fipronil	D	23	8	7,600	1,962 – 12,458	22	235	106 – 742
4. Chlorpyriphos	AD	20	7	16,450	5,618 – 21,068	20	66	38 – 101
5. Piperonyl butoxide	AD	19	10	6,397	2,448 – 12,458	17	124	60 – 560
6. Propiconazol	AD	19	1	167	NA	19	14	9 – 23
7. Iodocarb / IBPC	D	18	15	250	164 – 701	9	21	14 – 35
8. Tebuconazole	AD	18	0	NA	NA	18	10	7 – 14
9. Lindane	X	13	12	8,369	4,978 – 18,711	9	18	17 – 43
10. Azaconazole	X	10	3	337	217 – 351	10	23	12.18 – 131
ZONE 2 (66 households)								
1. Orthophenylphenol[d]	D	55	53	1,770	1,058 – 2,694	34	11	6 – 20
2. Lindane	X	28	21	4,137	1,886 – 11,736	20	19	11 – 24
3. Piperonyl butoxide	AD	23	17	1,635	866 – 8,947	17	91	59 – 315
4. Pentachlorophenol	X	22	12	172	72 – 292	17	13	4 – 30
5. Iodocarb / IBPC	D	20	18	488	168 – 649	10	10	6 – 19
6. Fipronil	D	19	5	3,656	789 – 11,352	19	69	30 – 230
7. Propiconazol	AD	15	1	89	NA	14	14	7 – 16
8. Permethrin	D	11	8	28,716	8,514 – 164,310	8	4,805	2,026 – 14,525
9. Anthraquinone	X	9	2	3,531	2,891 – 4,170	7	13	12 – 16
10. Tolyfluanide	X	9	9	18,855	5,291 – 30,014	5	32	18 – 63
ZONE 3 (68 households)								
1. Orthophenylphenol[d]	D	56	52	1,366	765 – 2,275	30	11	7 – 26
2. Tebuconazole	AD	38	2	119	109 – 130	37	12	7 – 20
3. Cymoxanil	AD	33	28	413	197 – 708	25	12	7 – 20
4. Fluazinam	A	32	26	184	119 – 290	21	9	5 – 15
5. Piperonyl butoxide	AD	30	19	4,425	1299 – 25,589	26	112	77 – 485
6. Dimetilan	X	28	7	146	110 – 833	27	10	6 – 16
7. Spiroxamine	A	28	0	NA	NA	28	12	7 – 20
8. Folpet	A	23	22	206,926	113,829 – 331,361	9	1,350	430 – 4,450
9. Lindane	X	23	18	12,169	4,377 – 19,769	18	15	10 – 27
10. Iodocarb / IBPC	D	22	22	194	158 – 352	4	11	10 – 16
ZONE 4 (36 households)								
1. Orthophenylphenol[d]	D	23	17	6,869	2,944 – 10,274	17	26	13 – 59
2. Pentachlorophenol	X	16	16	327	111 – 649	5	9	8 – 12
3. Iodocarb / IBPC	D	11	10	317	188 – 962	5	14	7 – 19
4. Piperonyl butoxide	AD	10	4	2,607	856 – 4,882	10	59	33 – 301
5. Lindane	X	9	8	798	592 – 1,299	4	9	4 – 44
6. DDT pp'	X	7	5	385	308 – 981	6	79	25 – 230
7. DDE pp'	X	6	5	192	192 – 558	5	7	4 – 23
8. DDT op'	X	6	5	346	231 – 750	5	16	8 – 45
9. DDD pp'	X	4	1	115	NA	4	12	6 – 38
10. Azinphos ethyl	X	3	0	NA	NA	3	15	11 – 17

[a]: *"A" agricultural use only; "D" domestic use only; "AD" mixed use (A and D); "X" Banned.* [b]*: occurrence (detection in either dust trap or floor wipe).* [c]*: ND "number of detection".* [d]*: Orthophenyphenol was found in two dust trap blanks performed in Zone 3, but not in other blanks.*

c) Comparison of recent and old dust samples

We observed pesticides more frequently in ODS than in RDS (2051 detections versus 1327 occurrences), especially in Zone 1 (830 versus 341). Overall, the distribution frequency significantly differed between RDS and ODS ($p<0.001$) with banned ($p<0.001$) and agricultural pesticides ($p<0.001$) being more frequent in ODS (Figure 3.5).



The figure presents the proportion of occurrences (recent dust) and detections (old dust) of pesticides summed across all households, per zone and per class. For recent dust samples, we considered one occurrence when pesticides were detected either in floor wipes or dust trap. Total occurrence/detection is presented above each column.

Figure 3.5: Proportion of pesticides frequency of detection, by type of use, type of dust, and by zone

The frequency distribution of pesticides differed between all zones ($p<0.001$) and between the three agricultural zones ($p<0.001$), in RDS and ODS. The proportion of

agricultural and mixed-usage pesticides combined significantly differed between zones for RDS (44%, 21%, and 52% versus 10%, respectively, $p<0.001$) and ODS (58%, 37%, and 50% versus 32%, respectively, $p<0.001$). We detected 36 additional pesticides (11 insecticides, 11 herbicides, 12 fungicides, 2 other) in ODS samples compared to RDS (Table S1). Among these compounds, 13 were banned in 2012, 18 limited to agricultural use and five had mixed usage. Twenty pesticides (six insecticides, nine herbicides, and five fungicides) detected in RDS were not found in ODS. The highest diversity of compounds was found in Zones 1 and 3 for ODS (99 and 95 compounds, respectively) and RDS (82 and 89 compounds, respectively).

The 10 most frequent compounds per zone in ODS are presented in Supplemental Materials (Supplemental Materials, Figure S1). Overall, 14 compounds (50%) among the top 10s in RDS were also among the top 10s in ODS. The overlap was 40%, 70%, 80%, and 30% for Zones 1, 2, 3, and 4, respectively. Compared to RDS, overlapping pesticides tended to have higher detection rate in ODS for Zones 1 and 2, similar detection rate in Zone 3 (except folpet: 90% in ODS versus 34% in RDS), and lower detection rate in Zone 4. Orthophenylphenol was less frequent in ODS in all zones (6 – 32% versus 49 – 84%).

d) Agricultural pesticides

In RDS, we detected 20 of the 29 expected agricultural pesticides, 10 of 16 for orchards, 9 of 10 for cereals and 11 of 13 for vineyards. Three additional orchard pesticides (cyproconazole, pyraclostrobin, tau-fluvalinate) and one cereal pesticide (sulcotrione) were found in ODS.

Agricultural pesticides were more frequent in zone 3 compared to zones 1 and 2 ($p<0.001$). Figure 3.6a shows the projection of households and pesticides on the first two axes of the PCoA of RDS. The first axis (explaining most of the dissimilarity) accounted for 33% of the total variation, in terms of dissimilarity of exposure profile between

households, and strongly contrasted Zone 3 (right side) with Zones 1 and 2 (left side). Ten fungicides out of 18 appeared on the right side of the plot and were therefore likely to be more common in households of Zone 3. Those included fluazinam, spiroxamine and folpet: three agricultural compounds among the ten most common pesticides in Zone 3 (see Table 3.5). Agricultural herbicides were all plotted on the left side of the graph (Zone 1 and 2), while insecticides and fungicides were distributed homogeneously. The second axis accounted for 8% of the total variation and did not reveal a clear contrast between zones. In ODS, the PCoA revealed contrast between the 3 zones (Figure 3.6b). Overall, according to reported usage, expected pesticides were associated to the corresponding zones, except 2 in RDS (boscalid and dimetomorph), and 4 in ODS (boscalid, pyraclostrobin, dimetomorph, and sulcotrione).

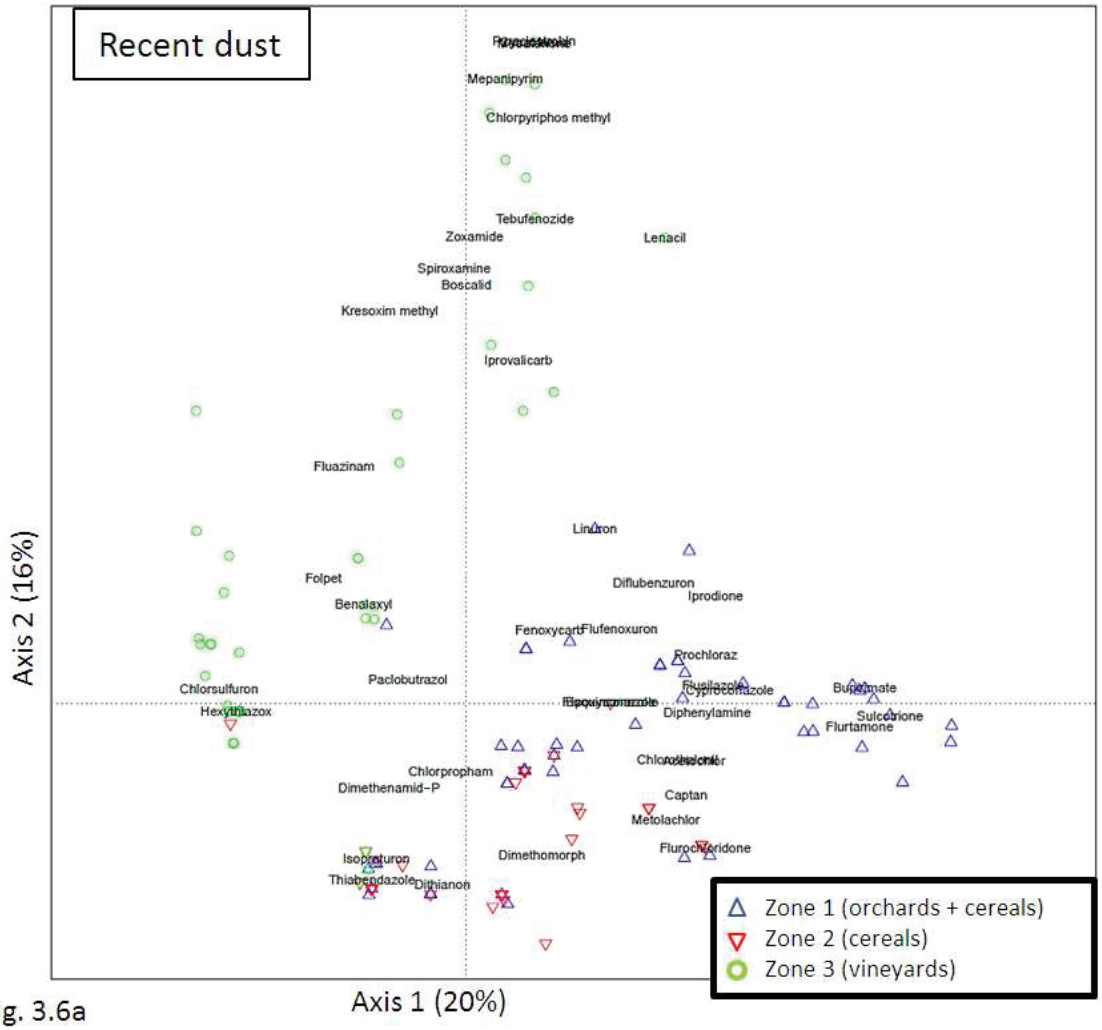


Fig. 3.6a

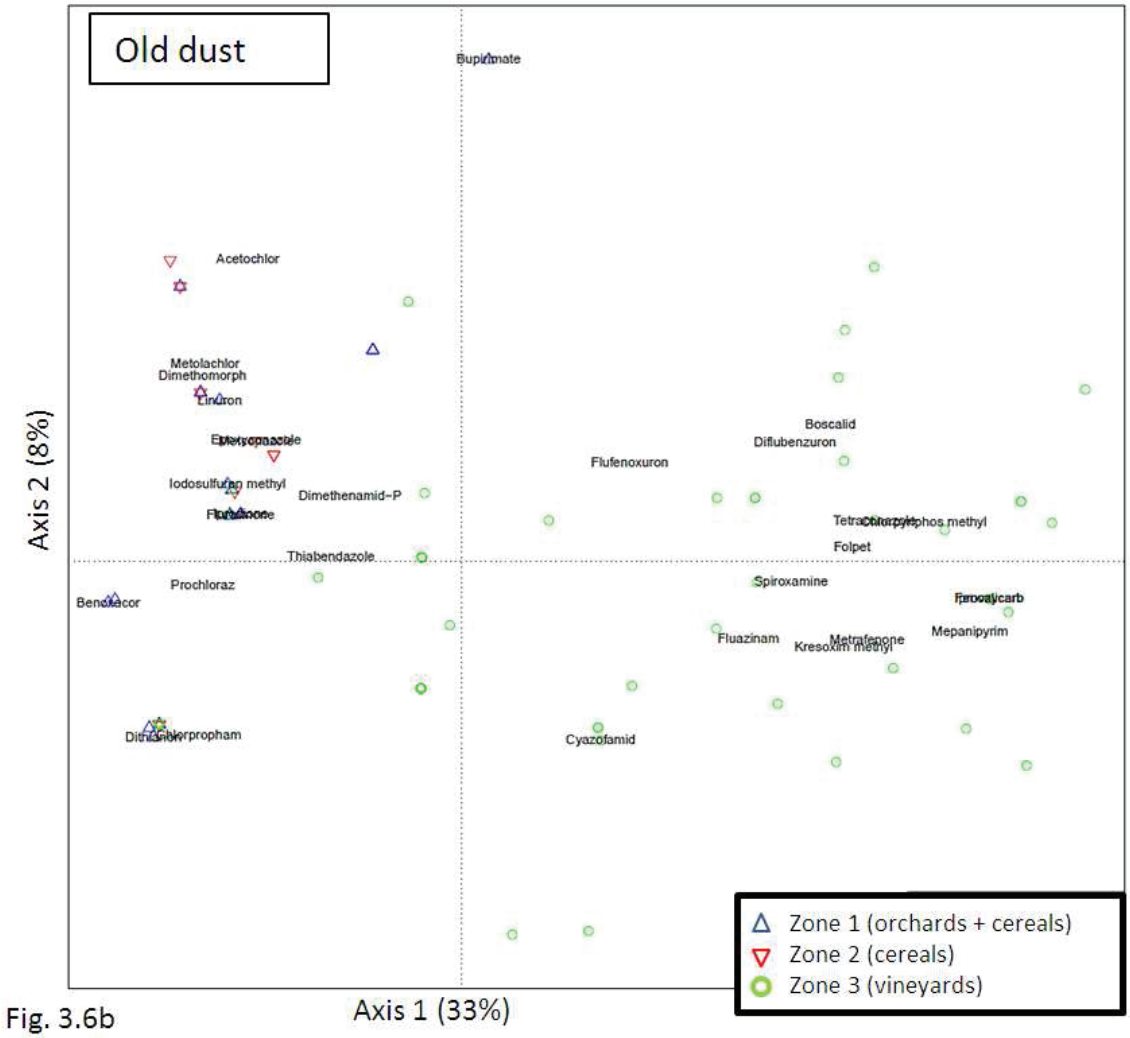


Fig. 3.6b

*PCoA biplot (households and pesticides) based on simple matching coefficient of dissimilarity between households in terms of profile of agricultural pesticides detected (presence/absence) in recent dust (**Fig 3.a**) and old dust (F**ig3.b**). The closer the households to each other in the graph (marks), the more similar their contamination profile. The variation explained by axis 1 and axis 2 is given in percentage of the total variation of dissimilarity between households. Pesticide names were* a posteriori *projected as weighted averages. **Fig3.a** includes 30 pesticides restricted to agricultural use detected in recent dust samples of 84 households (25 in Zone 1, 12 in Zone 2 and 47 in Zone 3). **Fig3.b** includes 43 pesticides restricted to agricultural use detected in old dust samples of 155 houses (52 in zone 1, 40 in zone 2 and 63 in zone 3).*

Figure 3.6: Main trends in households' similarity in terms of agricultural pesticides in recent dust and old dust

III.3.4. Discussion

To our knowledge, this is one of the largest studies reporting indoor pesticide contamination related to current or past agricultural practices and domestic uses in households from different agricultural and urban areas. The strength of our study relies on the number of targeted compounds (more than 400) measured simultaneously in recent (RDS) and cumulative (ODS) dust samples from 239 homes. Consistently with previous studies having targeted much fewer pesticides (Colt et al. 2004; Mercier et al. 2011;Obendorf et al. 2006; Quiros-Alcala et al. 2011; Tulve et al. 2006; Stout et al. 2009), we found contamination of indoor dust by historical and current domestic and agricultural pesticides uses.

Orthophenyphenol, a domestic fungicide used as conservative and disinfectant, was the most frequent pesticide in RDS in our study (70% of homes) as in the Colt et al.(2004) study (99%), a Californian study analyzing 30 pesticides in carpet dust of 513 study Californian homes. However, Colt et al (2006) reported propoxur, a household insecticide and 2,4-chlorphenoxyactetic acid, a herbicide with mixed-usage, as two of the most frequently detected pesticides in their study, while we found both in less than 5% of households in our study. Observed differences might be due to variation in agricultural and domestic practices and to sampling techniques.

Furthermore, Fipronil, banned from French agricultural use in 2005 but still authorized for pets, and piperonil butoxide (PB), a pesticide synergist (mixed-usage) often combined with pyrethroids in France, were respectively detected in 24% and 31% of our RDS. Our findings are comparable to those reported in a study designed to enhance the understanding of current levels of selected contaminants in dust wipe samples from 500 randomly selected residential homes in the USA (Stout et al. 2009). Results of that study included detection of fipronil and PB in 40% and 52% of 478 and 475 households, respectively, with similar surface loading (SL) for PB but lower SL for fipronil compared to our study. Tulves et al. (2006) found fipronil and PB in 8% and 23%, respectively, of

floor wipe samples from 168 US child care centers. However, they found much lower SL for both compounds compared to our study.

We found PCoA to be pertinent to analyze the spatial distribution of pesticides across households. PCoA showed clear clustering of zones in terms of agricultural pesticides profiles (Figure 3), suggesting an influence of agricultural practices on indoor contamination. PCoA also showed that agricultural pesticides expected for peaches/apricots, cereals/corn, and vineyards (Table S2) were predominantly detected in households from the corresponding zones. However, in French agriculture, multiple crops are grown in relatively small geographical areas, explaining the detection of several agricultural pesticides other than those expected. Also, similar cereal surfaces in zones 1 and 2, contribute to the similarity between the two zones. Conversely, households of zone 3, where vineyards were the predominant crop, differed in terms of agricultural pesticides detected. Even though not expected (Table S2), fluazinam, a fungicide authorized on vineyards, was the most frequent agricultural pesticide in our study, and almost exclusively detected in dust of homes in Zone 3 (Table S1), where vineyards are the predominant crop. To our knowledge, this study is the first to report measured values of fluazinam in house dust. Furthermore, Gunier et al. (2011) analyzed carpet dust samples of 89 Californian households for seven agricultural pesticides and reported detection rates between 34% and 96%. Only one, chlorpyrifos, was expected to be used on crops in our study area for peaches/apricots and grains (Table S2). We detected chlorpyrifos in RDS and ODS of 29 % and 62% households respectively in zone 1 (Table S1), but not in zone 2. In zone 3, although not expected on vineyards, we detected chlorpyrifos in 10% of RDS and 16% of ODS as well as chlorpyrifos-methyl in 19% of RDS and 6% of ODS. Also, we detected four additional compounds analyzed in the California study (carbaryl, diazinon, iprodione, and simazine), but each at a frequency < 5%. Differences in target crops and pesticide use practices between the Central Valley, California and the French Rhône-Alpes region probably explain these observations. However, the findings of our study and the study reported by Gunier et al (2011) suggest a potential role of pesticide drift deserving further examination. Overall, we detected (in RDS and ODS), 23 out of 29 pesticides expected to be used in agriculture during the sampling period. Non-detected

agricultural pesticides might have been metabolized before analyses, not been used during the sampling period, not been transported indoor, or present in our dust samples, but below laboratory detection limits.

Banned pesticides represented the predominant category in terms of overall occurrence. This observation is reported for the first time on such a large number of pesticides. Similar to Colt et al. (2004), pentachlorophenol, a banned wood preservative, was the second most frequent pesticide in our study. Conversely, lindane the second frequent banned pesticides in our study, was found only in few Californian homes in the study reported by Colt et al. (2006). The frequency of banned pesticides in RDS indicates on-going contamination. This may result from continued use, or more likely from continuous reemission from environmental or domestic sources, e.g. soil and construction material, as well as old indoor dust (Mercier et al. 2011). Our observations from the urban area (zone 4) support this hypothesis: despite a "zero-pesticide-use" policy since 2008, seven of the ten most frequent pesticides found in RDS in urban households were banned. However, our interpretation is limited by the lack of historical information on pesticide application and precise dates of restrictions for agricultural and domestic use. Similar to our study, permethrin was the most frequent detected pyrethroid insecticide in three other studies (Quiros-Alcala et al. 2011; Tulve et al. 2006; Colt et al. 2004).

Overall frequency of pesticides was higher in ODS compared to RDS with significantly different pesticides profiles, in particular a significantly higher rate of banned pesticides in ODS, probably explained by the longer time period covered by ODS. When comparing pesticides measured in dust traps versus floor wipe samples, we observed overall similar frequency of pesticides, but higher SLs for most pesticides in the dust trap samples even when considering that durations of dust collection differed (30 vs 7 days, respectively). For five pesticides with greater than 5% detection in one of the three zones (chlorpyrifos, diazinon, metolachlor, pendimethalin, tetramethrin), we compared our observations to findings reported by Obendorf et al. (2006) comparing SL for 17 pesticides on four media: carpet, a hard surface table and floor (using a wet filter paper), and a dry filter paper placed in a Petri dish for 7 days. The floor wipes (active sampling) and the filter

paper in the Petri dish (passive sampling) were close to our study. For two pesticides (diazinon, pendimethalin), Obendorf reported higher SL for the passive sampling technique, whereas we found higher SL for all five compounds in dust traps samples. Yet, the wipes and duration of dust collection differed between both studies; therefore comparisons should be made with caution. Also, it is likely that sampling methods, including wipe properties as well as surface characteristics influence the collection of pesticides (Mercier et al. 2011; Cettier et al. 2014). Placed higher above the floor, dust traps might have retained smaller particles and different pesticides than floor wipes (Edwards et al. 1998, Obendorf et al. 2006). Also, frequency distribution of insecticides, herbicides and fungicides significantly differed between dust trap and floor wipe samples in our study. Physico-chemical properties of pesticides and dust composition may further influence the adsorption of pesticides on dust and their deposition in the indoor environment (Obendorf et al. 2006, Cettier et al. 2014).

Our study has several limitations. While surface wiping and dust traps are appealing methods to collect indoor dust in large-scale studies, small quantities of sampled dust hindered us to report pesticides concentrations per dust quantity in our study and may explain low detection frequencies for some compounds. Overall, SL in our study remained below SL in studies using similar methods (Obendorf et al. 2006; Tulve et al.2006; Stout et al. 2009). Also, Obendorf et al (2006) found much lower SL in smooth floor, flat surface and dust trap samples compared to carpet dust, a long term repository. Furthermore, sampling periods, seasonal parameters and environmental factors may influence the pesticides frequency and concentration in indoor dust and warrant further investigation.

III.3.5. Conclusions

We identified 156 distinct pesticides in household dust from different agricultural areas, mainly at low SL and low detection rate. Indoor contamination appeared related to agricultural practices and domestic uses, while banned pesticides persist indoors and

contribute substantially to indoor contamination. The high detection rate of domestic pesticides stresses the need for improved public information and prevention strategies. Disparities between expected and detected pesticides suggest limitations of crop-pesticide matrices for assessing dust-borne indoor exposure proximate to fields. To avoid exposure misclassification bias, domestic pesticide exposure should be characterized in future epidemiology studies. Future research is needed to study potential health effects from pesticides in house dust, including the effects of cumulative exposures to mixtures of pesticides residues.

Acknowledgments

The authors acknowledge Elodie Faure (Centre Léon Bérard) for the GIS analyses, Kevin Saout for collecting part of the samples, and Helen Bailey (IARC) for proofreadings; all the volunteers that participated to the study; the departmental agricultural chambers, the farmers and the pesticide vendors that participate to the study. Rémi Béranger holds a doctoral grant from the *Région Rhône-Alpes*. This project was granted by the *Fondation de France* (Engt 2011-00023939) and the *Région Rhône-Alpes* (ref. 12-021795-01).

III.3.6 Supplemental Materials

Agricultural and domestic pesticides in housedust from different agricultural areas in France

Rémi Béranger, Elise Billoir, John R Nuckols, Jeffrey Blain, Joachim Schüz, Thierry Philip, Bruno Combourieu, Béatrice Fervers

Laboratory methods used for pesticides quantification

Upon receipt at the laboratory, the flasks (samples and blanks) were opened in a fume hood for 10 hours to allow evaporation of isopropanol. After adding 150 mL of dichloromethane and 100 µL of internal extraction standards (hexabromobenzene (2 mg/L) and triphenylphosphate (10 mg/L), Restek, Bellefonte, PA, USA), flasks were placed on a platform shaker for 4 hours. The solution was filtered through a funnel filled with glass wool saturated with sodium sulphate (99% purity, Chemlab, Zedelgen Belgium). One milliliter of iso-octane (99.5% purity, Carlo Erba Reagents, Milano, Italy) was added to the filtrate. The filtrate was concentrated in a TurboVap® evaporator workstation (TurboVap® II Zymark, Sotax, Allschwil, Switzerland) under nitrogen stream at 35°C to a final volume of 0.5 mL, and then adjusted to 1 mL with ethyl acetate (99.8% purity, Carlo Erba Reagents, Milano, Italy).

Two aliquots of 0.45 mL were made from the final solution. The first one was purified on magnesium silicate cartridges (CHROMABOND® Florisil®, Macherey-Nagel, Düren, Germany) with methanol (99.9% purity, Carlo Erba, Milano, Italy) for column conditioning, hexane (95% purity, Carlo Erba Reagents, Milano, Italy) and ethyl acetate as elution solvents. Chrysene D12 was added as internal standard. The sample was analyzed by gas chromatography (GC) (Varian-GC 450, SGE, Ringwood, Victoria, Australia) coupled with a mass spectrophotometer (MS) (Varian Saturn 2000, SGE, Ringwood, Victoria, Australia). The second aliquot was evaporated to dryness, resuspended in 450µL of (1:1) water/acetonitrile solution acidified with 0.1 % formic acid for injection, and then analysed using a high performance liquid chromatography (HPLC; Agilent 1100, Agilent Technologies, Waldbronn, Germany) coupled with a tandem MS (API4000, AB Sciex, Foster City, CA. USA). The limit of detection by both methods was 1ng/mL for all compounds. For all samples, extractions of internal standards were within 20 % the expected concentration. Analyses were performed in accordance with international quality standards (ISO-17025, http://www.iso.org/).

Table S1: List of compounds analyzed and the corresponding frequency of detection in recent and old dust, per zone

Name of the compound (n=417)	CAS	Occurrence[a] in recent dust					Detection in old dust				
		Zone 1 (69 houses)	Zone 2 (66 houses)	Zone 3 (68 houses)	Zone 4 (36 houses)	**TOTAL**	Zone 1 (69 houses)	Zone 2 (66 houses)	Zone 3 (68 houses)	Zone 4 (36 houses)	**TOTAL**
1-(3,4-dichlorophényl)-3-methylurea (DCPMU)	3567-62-2	0	2	5	1	**8**	10	8	6	3	**27**
1-(4-isopropylphényl)urea	34123-57-4	0	0	0	0	**0**	0	0	0	0	**0**
2,4 D	94-75-7	2	0	0	0	**2**	0	1	1	0	**2**
2,6 Dichlorobenzamid	2008-58-4	0	0	0	0	**0**	0	0	0	0	**0**
2,4 D- isopropyl-ester	94-11-1	0	0	0	0	**0**	0	0	0	0	**0**
2,4 D-methyl-ester	1928-38-7	0	0	0	0	**0**	0	0	0	0	**0**
2,4,5-T	93-76-5	0	0	0	0	**0**	0	0	0	0	**0**
2,4-DB	94-82-6	0	0	0	0	**0**	0	0	0	0	**0**
2,4-MCPB	94-81-5	0	0	0	0	**0**	0	0	0	0	**0**
3,4-dichlorophenylurea (DCPU)	2327-02-8	0	0	3	0	**3**	4	2	3	1	**10**
Acetamiprid	135410-20-7	0	0	1	0	**1**	1	0	0	0	**1**
Acetochlor	34256-82-1	3	2	0	0	**5**	21	14	2	1	**38**
Acibenzolar	126448-41-7	0	0	0	0	**0**	0	0	0	0	**0**
Acifluorfen	50594-66-6	0	0	0	0	**0**	0	0	0	0	**0**
Aclonifen	74070-46-5	2	0	0	0	**2**	1	0	0	0	**1**
Acrinathrin	101007-06-1	0	0	0	0	**0**	0	0	0	0	**0**
Alachlor	15972-60-8	0	0	0	0	**0**	0	0	0	0	**0**
Aldicarb	116-06-3	0	0	0	0	**0**	0	0	0	0	**0**
Aldrin	309-00-2	0	0	0	0	**0**	0	0	0	0	**0**

Name of the compound (n=417)	CAS	Occurrence[a] in recent dust					Detection in old dust				
		Zone 1 (69 houses)	Zone 2 (66 houses)	Zone 3 (68 houses)	Zone 4 (36 houses)	**TOTAL**	Zone 1 (69 houses)	Zone 2 (66 houses)	Zone 3 (68 houses)	Zone 4 (36 houses)	**TOTAL**
Allethrin	584-79-2	0	0	0	0	**0**	0	0	0	0	**0**
Alphamethrin	67375-30-8	0	2	0	0	**2**	0	3	4	1	**8**
Ametryn	834-12-8	0	0	0	0	**0**	0	0	0	0	**0**
Amidosulfuron	120923-37-7	0	0	0	0	**0**	0	0	0	0	**0**
Amitraz	33089-61-1	0	0	0	0	**0**	0	0	0	0	**0**
Anthraquinone	84-65-1	3	9	7	2	**21**	39	22	18	11	**90**
Asulam	3337-71-1	0	0	0	0	**0**	0	0	0	0	**0**
Atrazine[d]	1912-24-9	1	0	0	0	**1**	0	0	0	0	**0**
Atrazine deisopropyl[b]	*1007-28-9*	*0*	*0*	*0*	*0*	***0***	*0*	*0*	*0*	*0*	***0***
Atrazine desethyl[b]	*6190-65-4*	*0*	*0*	*0*	*0*	***0***	*0*	*0*	*0*	*0*	***0***
Azaconazole	60207-31-0	10	5	4	1	**20**	26	18	15	2	**61**
Azamethiphos	35575-96-3	0	0	0	0	**0**	0	0	0	0	**0**
Azimsulfuron	120162-55-2	0	0	0	0	**0**	0	0	0	0	**0**
Azinphos-ethyl	2642-71-9	0	0	11	3	**14**	0	0	16	0	**16**
Azinphos-methyl	86-50-0	3	2	6	0	**11**	9	2	1	0	**12**
Azoxystrobin	131860-33-8	0	0	1	0	**1**	5	0	8	1	**14**
Benalaxyl[e]	71626-11-4	0	0	0	0	**0**	0	0	1	0	**1**
Bendiocarb[d]	22781-23-3	2	3	0	0	**5**	0	0	0	0	**0**
Benfluraline	1861-40-1	0	0	0	0	**0**	0	0	0	0	**0**
Benfuracarbe	82560-54-1	0	0	0	0	**0**	0	0	0	0	**0**
Benomyl	17804-35-2	0	0	0	0	**0**	0	0	0	0	**0**
Benoxacor[d]	98730-04-2	1	0	0	0	**1**	0	0	0	0	**0**
Bentazon	25057-89-0	0	0	0	0	**0**	0	0	0	0	**0**

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Benthiocarbe (thiobencarb)	28249-77-6	0	0	0	0	**0**	0	0	0	0	**0**
Bensulfuron-methyl	83055-99-6	0	0	0	0	**0**	0	0	0	0	**0**
Beta-cyfluthrin	68359-37-5	0	0	0	0	**0**	0	0	0	0	**0**
Bifenazate	149877-41-8	0	0	0	0	**0**	0	0	0	0	**0**
Bifenox	42576-02-3	0	0	0	0	**0**	0	0	0	0	**0**
Bifenthrin	82657-04-3	3	6	4	0	**13**	10	6	4	0	**20**
Bioresmethrin	28434-01-7	0	0	0	0	**0**	0	0	0	0	**0**
Biphenyl	92-52-4	0	0	0	0	**0**	0	0	0	0	**0**
Bitertanol[e]	55179-31-2	0	0	0	0	**0**	2	0	0	0	**2**
Boscalid	188425-85-6	0	0	5	0	**5**	0	0	8	0	**8**
Brodifacoum[e]	56073-10-0	0	0	0	0	**0**	1	0	0	0	**1**
Bromacil	314-40-9	0	0	0	0	**0**	0	0	0	0	**0**
Bromadiolone	28772-56-7	0	0	0	0	**0**	0	0	0	0	**0**
Bromophos methyl	2104-96-3	0	0	0	0	**0**	0	0	0	0	**0**
Bromophos ethyl	4824-78-6	0	0	0	0	**0**	0	0	0	0	**0**
Bromopropylate	18181-80-1	0	0	0	0	**0**	0	0	0	0	**0**
Bromoxynil	1689-84-5	0	0	0	0	**0**	0	0	0	0	**0**
Bromoxynil octanoate	1689-99-2	0	0	0	0	**0**	0	0	0	0	**0**
Bromuconazole	116255-48-2	0	0	0	0	**0**	0	0	0	0	**0**
Bupirimate	41483-43-6	1	0	0	0	**1**	1	0	0	0	**1**
Buprofezin	953030-84-7	0	0	0	0	**0**	0	0	0	0	**0**
Butralin	33629-47-9	0	0	0	0	**0**	0	0	0	0	**0**
Buturon	3766-60-7	0	0	0	0	**0**	0	0	0	0	**0**

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Cadusafos	95465-99-9	0	0	0	0	**0**	0	0	0	0	**0**
Captafol	2425-06-1	0	0	0	0	**0**	0	0	0	0	**0**
Captan[e]	133-06-2	0	0	0	0	**0**	2	0	0	0	**2**
Carbaryl	63-25-2	2	1	2	0	**5**	2	4	2	0	**8**
Carbendazim	10605-21-7	1	0	1	0	**2**	43	8	13	1	**65**
Carbetamide[e]	16118-49-3	0	0	0	0	**0**	0	1	0	0	**1**
Carbofuran[d]	1563-66-2	1	0	0	0	**1**	0	0	0	0	**0**
Carbophenothion	786-19-6	0	0	0	0	**0**	0	0	0	0	**0**
Carbosulfan[d]	55285-14-8	1	0	0	0	**1**	0	0	0	0	**0**
Carfentrazone-ethyl	128639-02-1	0	0	0	0	**0**	0	0	0	0	**0**
Chinomethionat	2439-01-2	0	0	0	0	**0**	0	0	0	0	**0**
Chlorbromuron	13360-45-7	0	0	0	0	**0**	0	0	0	0	**0**
Chlorbufam[e]	1967-16-4	0	0	0	0	**0**	0	0	1	0	**1**
Chlordane	57-74-9	0	0	0	0	**0**	0	0	0	0	**0**
Chlordane alpha	5103-71-9	0	0	0	0	**0**	0	0	0	0	**0**
Chlordane Béta	5103-74-2	0	0	0	0	**0**	0	0	0	0	**0**
Chlordane Gamma	5566-34-7	0	0	0	0	**0**	0	0	0	0	**0**
Chlordecone	143-50-0	0	0	0	0	**0**	0	0	0	0	**0**
Chlorfenvinphos	470-90-6	1	0	0	0	**1**	1	0	0	0	**1**
Chlorfluazuron	71422-67-8	0	0	0	0	**0**	0	0	0	0	**0**
Chloridazon (pyrazon)[d]	1698-60-8	0	0	0	1	**1**	0	0	0	0	**0**
Chlormephos	24934-91-6	0	0	0	0	**0**	0	0	0	0	**0**
Chloroneb	2675-77-6	0	0	0	0	**0**	0	0	0	0	**0**

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Chlorophacinone	3691-35-8	0	0	0	0	**0**	0	0	0	0	**0**
Chlorothalonil[e]	1897-45-6	0	0	0	0	**0**	6	2	0	0	**8**
Chloroxuron	1982-47-4	0	0	0	0	**0**	0	0	0	0	**0**
Chlorpropham	101-21-3	8	5	4	1	**18**	10	8	8	0	**26**
Chlorpyriphos-ethyl	2921-88-2	20	0	7	0	**27**	43	0	11	3	**57**
Chlorpyriphos-methyl	5598-13-0	0	0	13	0	**13**	0	0	4	0	**4**
Chlorsulfuron[e]	64902-72-3	0	0	0	0	**0**	0	0	3	0	**3**
Chlorthal dimethyl	1861-32-1	0	0	0	0	**0**	0	0	0	0	**0**
Chlorthiamid	1918-13-4	0	0	0	0	**0**	0	0	0	0	**0**
Chlortoluron	15545-48-9	0	0	0	0	**0**	0	0	0	0	**0**
Cinidion-ethyl	142891-20-1	0	0	0	0	**0**	0	0	0	0	**0**
Clodinafop-propargyl	105512-06-9	0	0	0	0	**0**	0	0	0	0	**0**
Clofentezine[e]	74115-24-5	0	0	0	0	**0**	0	0	0	1	**1**
Clomazone	81777-89-1	0	0	0	0	**0**	0	0	0	0	**0**
Clopyralid	1702-17-6	0	0	0	0	**0**	0	0	0	0	**0**
Cloquintocet-mexyl	99607-70-2	0	0	0	0	**0**	0	0	0	0	**0**
Coumaphos	56-72-4	0	0	0	0	**0**	0	0	0	0	**0**
Coumatetralyl	5836-29-3	0	0	0	0	**0**	0	0	0	0	**0**
Cyanazine	21725-46-2	0	0	0	0	**0**	0	0	0	0	**0**
Cyazofamid	120116-88-3	0	0	1	0	**1**	0	0	1	0	**1**
Cycluron	2163-69-1	0	0	0	0	**0**	0	0	0	0	**0**
Cyfluthrin	68359-37-5	0	0	0	0	**0**	0	0	0	0	**0**
Cyhalofop-butyl	122008-85-9	0	0	0	0	**0**	0	0	0	0	**0**

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Cymoxanil	57966-95-7	0	0	33	0	**33**	0	0	6	0	**6**
Cypermethrin	52315-07-8	0	2	0	0	**2**	0	3	5	1	**9**
Cyproconazole[e]	94361-06-5	0	0	0	0	**0**	10	0	0	0	**10**
Cyprodinil	121552-61-2	1	0	15	0	**16**	17	1	4	0	**22**
DDT-op'	789-02-6	2	2	0	6	**10**	2	2	4	3	**11**
DDT-pp'	50-29-3	3	4	5	7	**19**	4	3	6	5	**18**
DDD-op[b]	*53-19-0*	*2*	*0*	*0*	*0*	***2***	*1*	*2*	*5*	*0*	***8***
DDD-pp[b]	*72-54-8*	*2*	*1*	*0*	*4*	***7***	*1*	*2*	*4*	*2*	***9***
DDE-op[be]	*3424-82-6*	*0*	*0*	*1*	*3*	***4***	*0*	*0*	*0*	*0*	***0***
DDE-pp[b]	*72-55-9*	*3*	*3*	*7*	*6*	***19***	*2*	*1*	*4*	*4*	***11***
Deltamethrin	52918-63-5	2	0	2	0	**4**	0	0	2	2	**4**
Demeton (O+S)	8065-48-3	0	0	0	0	**0**	0	0	0	0	**0**
Demeton-S-methyl	919-86-8	0	0	0	0	**0**	0	0	0	0	**0**
Déméton-S-methylsulphon	17040-19-6	0	0	0	0	**0**	0	0	0	0	**0**
Desmedipham	13684-56-5	0	0	0	0	**0**	0	0	0	0	**0**
Desmetryn	1014-69-3	0	0	0	0	**0**	0	0	0	0	**0**
Diallate	2303-16-4	0	0	0	0	**0**	0	0	0	0	**0**
Diazinon	333-41-5	3	1	3	1	**8**	2	1	3	0	**6**
Dicamba	1918-00-9	5	2	1	0	**8**	4	3	1	0	**8**
Dichlobenil[e]	1194-65-6	0	0	0	0	**0**	0	0	1	0	**1**
Dichlofenthion	97-17-6	0	0	0	0	**0**	0	0	0	0	**0**
Dichlofluanid	1085-98-9	0	0	3	0	**3**	0	0	1	0	**1**
Dichlorophen	97-23-4	1	2	3	0	**6**	14	12	21	1	**48**

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Dichlorprop[c]	120-36-5	0	0	0	0	**0**	0	0	0	0	**0**
Dichlorvos	62-73-7	0	0	0	0	**0**	0	0	0	0	**0**
Diclofop-methyl	51338-27-3	0	0	0	0	**0**	0	0	0	0	**0**
Dicofol	115-32-2	1	1	0	0	**2**	1	0	0	0	**1**
Dieldrin[d]	60-57-1	0	1	4	0	**5**	0	0	0	0	**0**
Diethofencarb	87130-20-9	0	0	0	0	**0**	0	0	0	0	**0**
Difenoconazole	119446-68-3	0	0	2	0	**2**	2	2	5	0	**9**
Difethialone	104653-34-1	0	0	0	0	**0**	0	0	0	0	**0**
Diflubenzuron	35367-38-5	3	0	17	0	**20**	35	0	14	0	**49**
Diflufenican	83164-33-4	1	0	0	0	**1**	1	0	0	0	**1**
Dimefuron	34205-21-5	0	0	0	0	**0**	0	0	0	0	**0**
Dimetachlor	50563-36-5	0	0	0	0	**0**	0	0	0	0	**0**
Dimethenamid[c]	87674-68-8	4	0	2	0	**6**	1	4	3	0	**8**
Dimethoate	60-51-5	0	0	0	0	**0**	0	0	0	0	**0**
Dimethomorph	110488-70-5	0	2	0	0	**2**	0	2	0	0	**2**
Dimetilan	644-64-4	4	0	28	0	**32**	37	0	43	1	**81**
Diniconazole	83657-24-3	0	0	0	0	**0**	0	0	0	0	**0**
Dinocap	39300-45-3	0	0	0	0	**0**	0	0	0	0	**0**
Dinoseb	88-85-7	0	0	0	0	**0**	0	0	0	0	**0**
Dinoterb[d]	1420-07-1	0	0	0	1	**1**	0	0	0	0	**0**
Diphenylamine	122-39-4	0	0	0	2	**2**	8	0	0	0	**8**
Disulfoton	298-04-4	0	0	0	0	**0**	0	0	0	0	**0**
Dithianon	3347-22-6	1	0	0	0	**1**	2	0	0	0	**2**

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Diuron	330-54-1	7	0	3	0	**10**	3	0	0	4	**7**
DNOC	534-52-1	2	5	10	0	**17**	7	17	16	1	**41**
Dodemorph	1593-77-7	0	0	0	0	**0**	0	0	0	0	**0**
Endosulfan alpha	959-98-8	0	0	1	0	**1**	0	2	3	0	**5**
Endosulfan beta	33213-65-9	0	0	1	0	**1**	0	2	2	0	**4**
Endosulfan sulfate	1031-07-8	0	0	1	0	**1**	0	1	1	0	**2**
Endrin[d]	72-20-8	0	0	1	0	**1**	0	0	0	0	**0**
Epoxiconazole	133855-98-8	0	1	0	0	**1**	0	1	0	0	**1**
EPTC	759-94-4	0	0	0	0	**0**	0	0	0	0	**0**
Esfenvalerate	66230-04-4	0	0	0	0	**0**	0	0	0	0	**0**
Ethidimuron	30043-49-3	0	0	0	0	**0**	0	0	0	0	**0**
Ethiofencarb	29973-13-5	0	0	0	0	**0**	0	0	0	0	**0**
Ethion (diethion)	563-12-2	0	0	0	0	**0**	0	0	0	0	**0**
Ethofumesate	26225-79-6	0	0	0	0	**0**	0	0	0	0	**0**
Ethoprophos	13194-48-4	0	0	0	0	**0**	0	0	0	0	**0**
Etofenprox	80844-07-1	0	0	0	0	**0**	0	0	0	0	**0**
Etoxazole	153233-91-1	0	0	0	0	**0**	0	0	0	0	**0**
Famoxadone	131807-57-3	0	0	0	0	**0**	0	0	0	0	**0**
Fenamidone	161326-34-7	0	0	0	0	**0**	0	0	0	0	**0**
Fenarimol	60168-88-9	0	0	0	0	**0**	0	0	0	0	**0**
Fenazaquin	120928-09-8	0	0	0	0	**0**	0	0	0	0	**0**
Fenbuconazole	114369-43-6	1	0	0	0	**1**	15	0	0	0	**15**
Fenchlorphos	299-84-3	0	0	0	0	**0**	0	0	0	0	**0**

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Fenhexamid	126833-17-8	0	0	0	0	**0**	0	0	0	0	**0**
Fenitrothion	122-14-5	0	0	0	0	**0**	0	0	0	0	**0**
Fenoxaprop-ethyl[c]	66441-23-4	0	0	0	0	**0**	0	0	0	0	**0**
Fenoxycarb	72490-01-8	0	0	1	0	**1**	3	0	1	0	**4**
Fenpropathrin	39515-41-8	0	0	0	0	**0**	0	0	0	0	**0**
Fenpropidin	67306-00-7	0	0	0	0	**0**	0	0	0	0	**0**
Fenpropimorph	67564-91-4	0	0	0	0	**0**	0	0	0	0	**0**
Fenpyroximate E[e]	134098-61-6	0	0	0	0	**0**	0	0	0	1	**1**
Fenthion	55-38-9	0	0	0	0	**0**	0	0	0	0	**0**
Fenuron[e]	101-42-8	0	0	0	0	**0**	0	1	0	0	**1**
Fipronil	120068-37-3	23	19	19	3	**64**	27	27	19	5	**78**
Flazasulfuron	104040-78-0	0	0	0	0	**0**	0	0	0	0	**0**
Fluazifop-p-butyl	79241-46-6	0	0	0	0	**0**	0	0	0	0	**0**
Fluazinam	79622-59-6	0	0	32	1	**33**	1	0	15	0	**16**
Fludioxonil	131341-86-1	2	0	6	0	**8**	19	1	10	0	**30**
Flufenacet (thiafluamide)	142459-58-3	0	0	0	0	**0**	0	0	0	0	**0**
Flufenoxuron	101463-69-8	0	3	6	0	**9**	27	21	17	0	**65**
Flumioxazin	103361-09-7	0	0	0	0	**0**	0	0	0	0	**0**
Flupyrsulfuron-methyl	144740-53-4	0	0	0	0	**0**	0	0	0	0	**0**
Fluquinconazole[e]	136426-54-5	0	0	0	0	**0**	0	1	0	0	**1**
Fluridone	59756-60-4	0	0	0	0	**0**	0	0	0	0	**0**
Flurochloridone[e]	61213-25-0	0	0	0	0	**0**	0	1	0	0	**1**
Fluroxypyr	69377-81-7	0	0	0	0	**0**	0	0	0	0	**0**

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Fluroxypyr-meptyl	81406-37-3	0	0	0	0	**0**	0	0	0	0	**0**
Flurprimidol	56425-91-3	0	0	0	0	**0**	0	0	0	0	**0**
Flurtamone	96525-23-4	1	0	0	0	**1**	8	0	0	0	**8**
Flusilazole[e]	85509-19-9	0	0	0	0	**0**	7	0	0	0	**7**
Flutolanil	66332-96-5	0	0	0	0	**0**	0	0	0	0	**0**
Flutriafol	76674-21-0	0	0	0	0	**0**	0	0	0	0	**0**
Folpet (folpel)	133-07-3	1	0	23	0	**24**	4	2	60	1	**67**
Fomesafen	72178-02-0	0	0	0	0	**0**	0	0	0	0	**0**
Fonofos	944-22-9	0	0	0	0	**0**	0	0	0	0	**0**
Formothion	2540-82-1	0	0	0	0	**0**	0	0	0	0	**0**
Fosthiazate	98886-44-3	0	0	0	0	**0**	0	0	0	0	**0**
Furalaxyl[d]	57646-30-7	0	0	1	0	**1**	0	0	0	0	**0**
Furathiocarb	65907-30-4	0	0	0	0	**0**	0	0	0	0	**0**
Haloxyfop[c]	69806-34-4	0	0	0	0	**0**	0	0	0	0	**0**
HCH alpha	319-84-6	0	0	1	0	**1**	3	3	1	0	**7**
HCH beta[e]	319-85-7	0	0	0	0	**0**	3	1	3	0	**7**
HCH delta	319-86-8	0	1	0	0	**1**	5	4	4	0	**13**
HCH epsilon[e]	6108-10-7	0	0	0	0	**0**	3	0	0	0	**3**
HCH gamma (lindane)	58-89-9	13	28	23	9	**73**	17	18	22	2	**59**
Heptachlor	76-44-8	0	0	0	0	**0**	0	0	0	0	**0**
Heptachlor epoxyde[b]	*1024-57-3*	*0*	*0*	*0*	*0*	***0***	*0*	*0*	*0*	*0*	***0***
Heptenophos	23560-59-0	0	0	0	0	**0**	0	0	0	0	**0**
Hexachlorobenzene	118-74-1	0	0	0	0	**0**	0	0	0	0	**0**

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Hexaconazole[e]	79983-71-4	0	0	0	0	**0**	2	0	0	0	**2**
Hexaflumuron	86479-06-3	0	0	1	0	**1**	0	0	1	0	**1**
Hexazinone	51235-04-2	0	0	0	0	**0**	0	0	0	0	**0**
Hexythiazox[e]	78587-05-0	0	0	0	0	**0**	0	0	1	0	**1**
Imazalil[d]	35554-44-0	1	0	0	0	**1**	0	0	0	0	**0**
Imazamethabenz-methyl	81405-85-8	0	0	0	0	**0**	0	0	0	0	**0**
Imidacloprid	138261-41-3	8	4	5	2	**19**	9	2	5	1	**17**
Indoxacarbe	173584-44-6	0	0	0	0	**0**	0	0	0	0	**0**
Iodocarb (IBCP)	55406-53-6	18	20	22	11	**71**	14	25	22	4	**65**
Iodofenphos	18181-70-9	0	0	0	0	**0**	0	0	0	0	**0**
Iodosulfuron-methyl[d]	144550-06-1	1	0	0	0	**1**	0	0	0	0	**0**
Ioxynil	1689-83-4	0	0	0	0	**0**	0	0	0	0	**0**
Ioxynil-methyl	3336-40-1	0	0	0	0	**0**	0	0	0	0	**0**
Ioxynil octanoate	3861-47-0	0	0	0	0	**0**	0	0	0	0	**0**
Iprodione	36734-19-7	1	0	0	0	**1**	5	1	2	0	**8**
Iprovalicarb	140923-17-7	0	0	1	0	**1**	5	0	12	0	**17**
Isazofos	42509-80-8	0	0	0	0	**0**	0	0	0	0	**0**
Isodrin	465-73-6	0	0	0	0	**0**	0	0	0	0	**0**
Isophenphos	25311-71-1	0	0	0	0	**0**	0	0	0	0	**0**
Isoproturon[e]	34123-59-6	0	0	0	0	**0**	0	1	0	0	**1**
Isoxaben[e]	82558-50-7	0	0	0	0	**0**	5	1	0	0	**6**
Isoxaflutole	141112-29-0	0	0	0	0	**0**	0	0	0	0	**0**
Kresoxim-methyl	143390-89-0	0	0	10	0	**10**	0	0	7	0	**7**

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Lamba-cyhalothrin	91465-08-6	4	0	1	0	**5**	3	1	0	0	**4**
Lenacil[e]	01/08/2164	0	0	0	0	**0**	0	0	1	0	**1**
Linuron	330-55-2	1	0	0	0	**1**	1	0	0	0	**1**
Lufenuron	103055-07-8	2	0	0	0	**2**	0	4	2	0	**6**
Malathion	121-75-5	2	0	0	0	**2**	2	0	0	0	**2**
MCPA-1-butyl ester	1713-12-8	0	0	0	0	**0**	0	0	0	0	**0**
MCPA-2-ethylhexyl ester	29450-45-1	0	0	0	0	**0**	0	0	0	0	**0**
MCPA-butoxyethyl ester	19480-43-4	0	0	0	0	**0**	0	0	0	0	**0**
MCPA-ethyl ester	2698-38-6	0	0	0	0	**0**	0	0	0	0	**0**
MCPA-methyl ester	2436-73-9	0	0	0	0	**0**	0	0	0	0	**0**
2,4-MCPA[d]	94-74-6	0	0	1	0	**1**	0	0	0	0	**0**
Mecoprop[c]	93-65-2	0	1	1	0	**2**	0	0	0	1	**1**
Mecoprop-1-octyl ester	161922-37-8	0	0	0	0	**0**	0	0	0	0	**0**
Mecoprop-2,4,4-trimethylpentyl ester	217487-13-3	0	0	0	0	**0**	0	0	0	0	**0**
Mecoprop-2-butoxyethyl ester	23359-62-8	0	0	0	0	**0**	0	0	0	0	**0**
Mecoprop-2-ethylhexyl ester[c]	71526-69-7	0	0	0	0	**0**	0	0	0	0	**0**
Mecoprop-2-octyl ester	28473-03-2	0	0	0	0	**0**	0	0	0	0	**0**
Mecoprop-methyl ester	2786-19-8	0	0	0	0	**0**	0	0	0	0	**0**
Mecoprop-n/iso-butyl ester	N/A	0	0	0	0	**0**	0	0	0	0	**0**
Mefenacet	73250-68-7	0	0	0	0	**0**	0	0	0	0	**0**
Mefenpyr-diethyl	135590-91-9	0	0	0	0	**0**	0	0	0	0	**0**
Mefluidide	53780-34-0	0	0	0	0	**0**	0	0	0	0	**0**

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Mepanipyrim	110235-47-7	0	0	3	0	**3**	0	0	1	0	**1**
Mepronil	55814-41-0	0	0	0	0	**0**	0	0	0	0	**0**
Mercaptodimethur (methiocarb)	2032-65-7	0	0	0	0	**0**	0	0	0	0	**0**
Mesosulfuron-methyl	208465-21-8	0	0	0	0	**0**	0	0	0	0	**0**
Mesotrione	104206-82-8	0	0	0	0	**0**	0	0	0	0	**0**
Metalaxyl	57837-19-1	0	0	2	0	**2**	0	0	1	0	**1**
Metamitron	41394-05-2	0	0	0	0	**0**	0	0	0	0	**0**
Metazachlor	67129-08-2	0	0	0	0	**0**	0	0	0	0	**0**
Metconazole[d]	125116-23-6	0	1	0	0	**1**	0	0	0	0	**0**
Metabenzthiazuron	18691-97-9	1	0	0	0	**1**	2	0	0	0	**2**
Methidathion	950-37-8	0	0	0	0	**0**	0	0	0	0	**0**
Methomyl	16752-77-5	0	0	0	0	**0**	0	0	0	0	**0**
Methoxychlor[e]	72-43-5	0	0	0	0	**0**	2	1	0	0	**3**
Metobromuron	3060-89-7	0	0	0	0	**0**	0	0	0	0	**0**
Metolachlor	51218-45-2	6	3	0	0	**9**	9	9	0	0	**18**
Metosulam	139528-85-1	0	0	0	0	**0**	0	0	0	0	**0**
Metoxuron	19937-59-8	0	0	0	0	**0**	0	0	0	0	**0**
Metrafenone	220899-03-6	0	0	4	0	**4**	0	0	1	0	**1**
Metribuzin[d]	21087-64-9	1	0	0	0	**1**	0	0	0	0	**0**
Metsulfuron-methyl	74223-64-6	0	0	0	0	**0**	0	0	0	0	**0**
Mevinphos	7786-34-7	0	0	0	0	**0**	0	0	0	0	**0**
Mirex	2385-85-5	0	0	0	0	**0**	0	0	0	0	**0**
Molinate	2212-67-1	0	0	0	0	**0**	0	0	0	0	**0**

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Monolinuron	1746-81-2	0	0	0	0	**0**	0	0	0	0	**0**
Monuron	150-68-5	0	0	0	0	**0**	0	0	0	0	**0**
Myclobutanil	88671-89-0	5	0	1	0	**6**	3	0	0	0	**3**
Naled	300-76-5	0	0	0	0	**0**	0	0	0	0	**0**
Napropamide	15299-99-7	0	0	0	0	**0**	0	0	0	0	**0**
Naptalam	132-66-1	0	0	0	0	**0**	0	0	0	0	**0**
Neburon	555-37-3	0	0	0	0	**0**	0	0	0	0	**0**
Norflurazon	27314-13-2	0	0	0	0	**0**	0	0	0	0	**0**
Norflurazon desmethyl[b]	*23576-24-1*	*0*	*0*	*0*	*0*	***0***	*0*	*0*	*0*	*0*	***0***
Nuarimol	63284-71-9	0	0	0	0	**0**	0	0	0	0	**0**
Ofurace[d]	58810-48-3	0	0	1	0	**1**	0	0	0	0	**0**
Orthophenylphenol (2-phenylphenol)	90-43-7	34	55	56	23	**168**	12	21	10	2	**45**
Oryzalin	19044-88-3	3	0	3	0	**6**	39	5	15	0	**59**
Oxadiargyl	39807-15-3	0	0	0	0	**0**	0	0	0	0	**0**
Oxadiazon	19666-30-9	3	1	2	0	**6**	4	0	3	0	**7**
Oxadixyl	77732-09-3	0	0	0	0	**0**	0	0	0	0	**0**
Oxamyl	23135-22-0	0	0	0	0	**0**	0	0	0	0	**0**
Oxydemeton-methyl	301-12-2	0	0	0	0	**0**	0	0	0	0	**0**
Oxyfluorfen	42874-03-3	0	0	0	0	**0**	0	0	0	0	**0**
Paclobutrazol[e]	76738-62-0	0	0	0	0	**0**	4	2	8	3	**17**
Parathion-ethyl	56-38-2	0	0	0	0	**0**	0	0	0	0	**0**
Parathion-methyl	298-00-0	0	0	0	0	**0**	0	0	0	0	**0**
Penconazole	66246-88-6	0	0	0	0	**0**	0	0	0	0	**0**

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Pencycuron	66063-05-6	0	0	0	0	**0**	0	0	0	0	**0**
Pendimethalin	40487-42-1	9	1	0	0	**10**	16	3	0	0	**19**
Pentachlorobenzene	608-93-5	0	0	0	0	**0**	0	0	0	0	**0**
Pentachlorophenol	87-86-5	26	22	22	16	**86**	9	6	28	1	**44**
Permethrin	52645-53-1	9	11	19	3	**42**	11	20	25	0	**56**
Phenmedipham	13684-63-4	0	0	0	0	**0**	0	0	0	0	**0**
Phorate	298-02-2	0	0	0	0	**0**	0	0	0	0	**0**
Phosalone	2310-17-0	0	0	0	0	**0**	0	0	0	0	**0**
Phosmet	732-11-6	0	0	0	0	**0**	0	0	0	0	**0**
Phosphamidon	13171-21-6	0	0	0	0	**0**	0	0	0	0	**0**
Phoxim[e]	14816-18-3	0	0	0	0	**0**	0	0	0	1	**1**
Picolinafen	137641-05-5	0	0	0	0	**0**	0	0	0	0	**0**
Picoxystrobin	117428-22-5	0	0	0	0	**0**	0	0	0	0	**0**
Piperonil butoxide	51-03-6	19	23	30	10	**82**	21	20	26	2	**69**
Pirimicarb	23103-98-2	0	0	0	0	**0**	0	0	0	0	**0**
Pretilachlor	51218-49-6	0	0	0	0	**0**	0	0	0	0	**0**
Prochloraz	67747-09-5	1	1	0	0	**2**	4	1	0	0	**5**
Procymidone[e]	32809-16-8	0	0	0	0	**0**	0	0	8	0	**8**
Profenophos	41198-08-7	0	0	0	0	**0**	0	0	0	0	**0**
Promecarb	2631-37-0	0	0	0	0	**0**	0	0	0	0	**0**
Prometon	1610-18-0	0	0	0	0	**0**	0	0	0	0	**0**
Prometryn[d]	7287-19-6	0	0	1	0	**1**	0	0	0	0	**0**
Propachlor	1918-16-7	0	0	0	0	**0**	0	0	0	0	**0**

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Propanil	709-98-8	0	0	0	0	**0**	0	0	0	0	**0**
Propaquizafop	111479-05-1	0	0	0	0	**0**	0	0	0	0	**0**
Propargite	2312-35-8	0	0	0	0	**0**	0	0	0	0	**0**
Propazine	139-40-2	0	0	0	0	**0**	0	0	0	0	**0**
Propetamphos	31218-83-4	0	0	0	0	**0**	0	0	0	0	**0**
Propiconazole	60207-90-1	19	15	16	2	**52**	39	29	14	3	**85**
Propoxur	114-26-1	3	4	2	2	**11**	6	8	5	0	**19**
Propoxycarbazone-sodium	181274-15-7	0	0	0	0	**0**	0	0	0	0	**0**
Propyzamide	23950-58-5	0	0	0	0	**0**	0	0	0	0	**0**
Prosulfocarb	52888-80-9	0	0	0	0	**0**	0	0	0	0	**0**
Pyraclostrobin[e]	175013-18-0	0	0	0	0	**0**	0	0	1	0	**1**
Pyrazophos	13457-18-6	0	0	0	0	**0**	0	0	0	0	**0**
Pyridaben	96489-71-3	0	0	0	0	**0**	0	0	0	0	**0**
Pyridate	55512-33-9	0	0	0	0	**0**	0	0	0	0	**0**
Pyrifenox	88283-41-4	0	0	2	0	**2**	1	0	0	0	**1**
Pyrimethanil	53112-28-0	1	1	13	0	**15**	0	0	7	0	**7**
Pyrimiphos-ethyl	23505-41-1	0	0	0	0	**0**	0	0	0	0	**0**
Pyrimiphos-methyl	29232-93-7	0	0	0	0	**0**	0	0	0	0	**0**
Pyriproxyfen	95737-68-1	0	0	0	0	**0**	0	0	0	0	**0**
Quinalfos (chinalphos)[e]	13593-03-8	0	0	0	0	**0**	0	0	1	0	**1**
Quinoxyfen	124495-18-7	0	0	0	0	**0**	0	0	0	0	**0**
Quintozene	82-68-8	0	0	0	0	**0**	0	0	0	0	**0**
Quizalofop[c]	76578-12-6	0	0	0	0	**0**	0	0	0	0	**0**

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Quizalofop-ethyl	76578-14-8	0	0	0	0	**0**	0	0	0	0	**0**
Rotenone[e]	83-79-4	0	0	0	0	**0**	2	0	0	0	**2**
Sebuthylazine	7286-69-3	0	0	0	0	**0**	0	0	0	0	**0**
Secbumeton	26259-45-0	0	0	0	0	**0**	0	0	0	0	**0**
Silthiofam (silthiopham)	175217-20-6	0	0	0	0	**0**	0	0	0	0	**0**
Simazine	122-34-9	0	0	1	0	**1**	1	1	1	0	**3**
Spinosad	168316-95-8	0	0	0	0	**0**	0	0	0	0	**0**
Spiroxamine	118134-30-8	0	0	28	0	**28**	0	0	14	0	**14**
Sulcotrione[e]	99105-77-8	0	0	0	0	**0**	1	0	0	0	**1**
Sulfotep (sulfotepp)	3689-24-5	0	0	0	0	**0**	0	0	0	0	**0**
Tau-fluvalinate[e]	102851-06-9	0	0	0	0	**0**	6	0	0	0	**6**
Tebuconazole	107534-96-3	18	5	38	0	**61**	52	26	37	2	**117**
Tebufenozide[e]	112410-23-8	0	0	0	0	**0**	0	0	1	0	**1**
Tebufenpyrad	119168-77-3	0	0	0	0	**0**	0	0	0	0	**0**
Tebutam (butam)	35256-85-0	0	0	0	0	**0**	0	0	0	0	**0**
Teflubenzuron	83121-18-0	2	1	0	0	**3**	0	1	0	0	**1**
Temephos (temefos)	3383-96-8	0	0	0	0	**0**	0	0	0	0	**0**
Terbacil	5902-51-2	0	0	0	0	**0**	0	0	0	0	**0**
Terbufos	13071-79-9	0	0	0	0	**0**	0	0	0	0	**0**
Terbumeton	33693-04-8	0	0	0	0	**0**	0	0	0	0	**0**
Terbumeton-desethyl[b]	*30125-64-5*	*0*	*0*	*0*	*0*	***0***	*0*	*0*	*0*	*0*	***0***
Terbuthylazine	5915-41-3	0	0	1	0	**1**	1	0	0	0	**1**
Terbuthylazine-desethyl[b]	*30125-63-4*	*0*	*0*	*0*	*0*	***0***	*0*	*0*	*0*	*0*	***0***

Name of the compound (n=417)	CAS	Occurrence[a] in recent dust					Detection in old dust				
		Zone 1 (69 houses)	Zone 2 (66 houses)	Zone 3 (68 houses)	Zone 4 (36 houses)	**TOTAL**	Zone 1 (69 houses)	Zone 2 (66 houses)	Zone 3 (68 houses)	Zone 4 (36 houses)	**TOTAL**
Terbutryn (terbutryne)	886-50-0	0	0	1	2	**3**	1	5	7	1	**14**
Tetrachlorobenzene	12408-10-5	0	0	0	0	**0**	0	0	0	0	**0**
1,2,3,4-tetrachlorobenzene	634-66-2	0	0	0	0	**0**	0	0	0	0	**0**
1,2,4,5-tetrachlorobenzene	95-94-3	0	0	0	0	**0**	0	0	0	0	**0**
Tetrachlorvinphos	22248-79-9	1	0	0	0	**1**	1	0	1	0	**2**
Tetraconazole[d]	112281-77-3	0	0	1	0	**1**	0	0	0	0	**0**
Tetradifon	116-29-0	0	0	0	0	**0**	0	0	0	0	**0**
Tetramethrin	7696-12-0	2	2	7	1	**12**	1	1	2	0	**4**
Thiabendazole	148-79-8	4	0	2	1	**7**	2	0	0	0	**2**
Thiacloprid	111988-49-9	3	0	0	0	**3**	7	0	0	0	**7**
Thiazafluron (thiazasulfuron)	25366-23-8	0	0	0	0	**0**	0	0	0	0	**0**
Thifensulfuron-methyl	79277-27-3	0	0	0	0	**0**	0	0	0	0	**0**
Thiodicarb	59669-26-0	0	0	0	0	**0**	0	0	0	0	**0**
Thiometon	640-15-3	0	0	0	0	**0**	0	0	0	0	**0**
Tolclofos-methyl	57018-04-9	0	0	0	0	**0**	0	0	0	0	**0**
Tolylfluanid	731-27-1	4	9	11	2	**26**	15	22	21	1	**59**
Tralomethrin[d]	66841-25-6	0	0	1	0	**1**	0	0	0	0	**0**
Triadimefon[e]	43121-43-3	0	0	0	0	**0**	1	0	0	0	**1**
Triadimenol	55219-65-3	0	0	0	0	**0**	0	0	0	0	**0**
Triallate	2303-17-5	0	0	0	0	**0**	0	0	0	0	**0**
Triasulfuron[e]	82097-50-5	0	0	0	0	**0**	0	0	1	0	**1**
Triazamate	112143-82-5	0	0	0	0	**0**	0	0	0	0	**0**
Triazophos	24017-47-8	0	0	0	0	**0**	0	0	0	0	**0**

Name of the compound (n=417)	CAS	Occurrence[a] in recent dust					Detection in old dust				
		Zone 1 (69 houses)	Zone 2 (66 houses)	Zone 3 (68 houses)	Zone 4 (36 houses)	**TOTAL**	Zone 1 (69 houses)	Zone 2 (66 houses)	Zone 3 (68 houses)	Zone 4 (36 houses)	**TOTAL**
Triazoxide	72459-58-6	0	0	0	0	**0**	0	0	0	0	**0**
Trichlopyr[d]	55335-06-3	1	0	0	0	**1**	0	0	0	0	**0**
Trifloxystrobin	141517-21-7	0	0	11	0	**11**	0	0	8	0	**8**
Triflumuron	64628-44-0	0	0	1	0	**1**	1	0	1	0	**2**
Trifluralin	1582-09-8	1	0	0	0	**1**	2	0	0	0	**2**
Trinexapac-ethyl	95266-40-3	0	0	0	0	**0**	0	0	0	0	**0**
Vinclozolin	50471-44-8	0	0	0	0	**0**	0	0	0	0	**0**
Zoxamide[e]	156052-68-5	0	0	0	0	**0**	0	0	10	0	**10**

[a]: Occurrence is defined as the number of distinct compounds detected in each home, either from home's dust trap or floor wipe samples (if a pesticide was detected in both, we considered only one occurrence in the households). [b]: pesticide metabolite. [c]: There is existing isomer that cannot be differentiated using our technics of laboratory analyses. [d]: Detected only in recent dust samples. [e]: Detected only in old dust samples. Households were located less than 1000 meters from orchards (zone 1, n= 69), cereals (zone 2, n= 66), and vineyards (zone 3, n= 68), or in the urban area at least 2000m from any agricultural crops (zone 4, n= 36).

Table S2: List of agricultural pesticides expected to be used during sampling periods

	Peaches & apricots	*Corns & grains*	*Vineyards*
INSECTICIDES			
chlorpyriphos-ethyl	X	X	
cypermethrin		X	
deltamethrin	X		
esfenvalerate	X		
lambda Cyhalothrin	X		
tau Fluvalinate	X		
FONGICIDES			
boscalid	X		
bupirimate	X		
captane	X		
cyazofamid			X
cymoxanyl			X
cyproconazole	X		
cyprodinil			X
difenoconazole	X		X
dimetomorph			X
fenbuconazole	X		
fenhexamid			X
fludioxynil			X
folpet			X
indoxacarb	X		
kresoxym-methyl	X		X
metconazole		X	
myclobutanil	X		
penconazole	X		
pyraclostrobin	X		
tebuconazole		X	X
triadimenol			X
trifloxistobin			X
spiroxamine			X
HERBICIDES			
acetochlor		X	
dicamba		X	
dimethenamid		X	
S-metolachlor		X	
mesotrione		X	
sulcotrione		X	

Pesticides expected in April-May (peaches & apricots; corns & grains) or June-July (vineyards) in France in 2012. Based on information from departmental agricultural chambers, pesticide vendors, and famers.

Table S3: Proportion of agricultural fields within 1000 meters

Type of crops	Zone 1 (median + IQR[a])	Zone 2 (median + IQR[a])	Zone 3 (median + IQR[a])
Cereals[b]	21.6% (12.9 – 30.2)	21.5% (14.5 – 31.8)	2.5% (0 – 9.8)
Peaches & Apricots[b]	4.3% (2.0 - 10.5)	0	0
Vineyards[b]	0.1% (0 - 1.4)	0	29.7% (6.3 – 48.3)
Cereals, organic[c]	0% (0 – 0.4)	0% (0 – 1.2)	0
Fallow[c]	0.2% (0 – 0.7)	0.9% (0.1 – 1.8)	0% (0 – 0.2)
Leguminous[c]	0	2.7% (0 – 3.2)	0
Meadow[c]	2.9% (0.7 – 4.4)	5% (0 – 9.1)	7.4% (3.1 – 16.5)
Meadow, organic[c]	0	0% (0 – 0.3)	0% (0 – 0.3)
Other orchards[c]	0.6% (0 – 1.9)	0	0% (0 – 0.1)
Sunflower & rape[c]	4.5% (0.09 – 7.4)	3.6% (1.2 – 4.9)	0% (0 – 1.7)
Vegetables[c]	0.2 (0 – 2.8)	0	0
Vineyard, organic[c]	0	0	0% (0 – 0.8)
Various[c]	0.5% (0 - 2.2)	0% (0 – 0.2)	0.2% (0 – 0.6)
Undefined[c]	0.3% (0 – 0.5)	0.5% (0 – 1.3)	0

[a]: Interquartile range; [b]: Expected crops; [c]: Crops grouped under the label "other crops" in the Table 1 (main text).

Figure S1: Comparison of pesticide ranking in terms of occurrence, depending to the type of dust

Recent dust (wipes + traps)			Correspondence in ranking between recent and old dust	Old dust		
Name	Status	Occurrence		Name	Status	Detection
ZONE 1				ZONE 1		
Orthophenylphenol	D	34 (49%)	>20	Tebuconazole	AD	52 (75%)
Pentachlorophenol	X	26 (38%)	>20	Chlorpyriphos	AD	43 (62%)
Fipronil	D	23 (33%)	10	Carbendazim	X	43 (62%)
Chlorpyriphos	AD	20 (29%)	2	Oryzalin	AD	39 (56%)
Piperonyl butoxide	AD	19 (28%)	13	Propiconazol	AD	39 (56%)
Propiconazol	AD	19 (28%)	5	Anthraquinone	X	39 (56%)
Iodocarb / IBPC	D	18 (26%)	20	Dimetilan	X	37 (54%)
Tebuconazole	AD	18 (26%)	1	Diflubenzuron	A	35 (51%)
Lindane	X	13 (19%)	16	Flufenoxuron	A	27 (39%)
Azaconazole	X	10 (14%)	>20	Fipronil	D	27 (39%)
ZONE 2				ZONE 2		
Orthophenylphenol	D	55 (83%)	8	Propiconazol	AD	29 (44%)
Lindane	X	28 (42%)	12	Fipronil	D	27 (41%)
Piperonyl butoxide	AD	23 (35%)	9	Tebuconazole	AD	26 (39%)
Pentachlorophenol	X	22 (33%)	>20	Iodocarb / IBPC	D	25 (38%)
Iodocarb / IBPC	D	20 (30%)	4	Anthraquinone	X	22 (33%)
Fipronil	D	19 (29%)	2	Tolyfluanide	X	22 (33%)
Propiconazol	AD	15 (23%)	1	Flufenoxuron	A	21 (32%)
Permethrin	D	11 (17%)	10	Orthophenylphenol	D	21 (32%)
Anthraquinone	X	9 (14%)	5	Piperonyl butoxide	AD	20 (30%)
Tolyfluanide	X	9 (14%)	6	Permethrin	D	20 (30%)
ZONE 3				ZONE 3		
Orthophenylphenol	D	56 (84%)	>20	Folpet	A	60 (90%)
Tebuconazole	AD	38 (57%)	3	Dimetilan	X	43 (64%)
Cymoxanil	AD	33 (49%)	>20	Tebuconazole	AD	37 (55%)
Fluazinam	A	32 (48%)	>20	Pentachlorophenol	X	28 (42%)
Piperonyl butoxide	AD	30 (45%)	5	Piperonyl butoxide	AD	26 (39%)
Dimetilan	X	28 (42%)	2	Permethrin	D	25 (37%)
Spiroxamine	A	28 (42%)	20	Iodocarb / IBPC	D	22 (33%)
Folpet	A	23 (34%)	1	Lindane	X	22 (33%)
Lindane	X	23 (34%)	8	Dichlorophene	X	21 (31%)
Iodocarb / IBPC	D	22 (33%)	7	Tolyfluanide	X	21 (31%)
ZONE 4				ZONE 4		
Orthophenylphenol	D	23 (64%)	15	Anthraquinone	X	11 (31%)
Pentachlorophenol	X	16 (44%)	>20	Fipronil	D	5 (14%)
Iodocarb / IBPC	D	11 (31%)	4	DDT pp'	X	5 (14%)
Piperonyl butoxide	AD	10 (28%)	13	Iodocarb / IBPC	D	4 (11%)
Lindane	X	9 (25%)	18	DDE pp'	X	4 (11%)
DDT pp'	X	7 (19%)	3	Diuron	X	4 (11%)
DDE pp'	X	6 (17%)	5	Paclobutrazol	A	3 (8%)
DDT op'	X	6 (17%)	11	Chlorpyriphos	AD	3 (8%)
DDD pp'	X	4 (11%)	17	Propiconazol	AD	3 (8%)
Azinphos ethyl	X	3 (8%)	>20	DCPMU	X	3 (8%)

Ranking of pesticides (top 10) in a decreasing order of their occurrence in recent (left side) and detection old dust samples (right side). Status of pesticides: X = banned; A = agricultural use only; D = domestic use only; AD = mixed usage (A and D). For compounds present in both recent and old dust top 10, a dash indicates their respective position in the two ranking.

III.4 (article #4)

Environmental determinants of the indoor exposure to agricultural pesticides

Rémi Béranger[(1,2,3)$], Elise Billoir[(4)$], John R Nuckols[(5,6)], Elodie Faure[(1)], Jeffrey Blain[(1)], Virginie Chasles[(7)], Thierry philip[(1)], Joachim Schüz[(2)£], Béatrice Fervers[(1,3)£]

(1) Unité Cancer et Environnement, Centre Léon Bérard, Lyon, France

(2) Section of Environment and Radiation, International Agency for Research on Cancer (IARC), Lyon, France

(3) EAM 4128, Université Claude Bernard, Lyon, France

(4) Université de Lorraine, CNRS UMR 7360, Laboratoire Interdisciplinaire des Environnements Continentaux (LIEC), Metz, France

(5) Dept. of Environ and Radiol. Health Sci, Colorado State University, Fort Collins, CO, USA

(6) Principal, JRN Environmental Health Sciences, Ltd, North Bethesda, MD, USA

(7) Departement of Geographiy, EAM 4128, Université Jean Moulin, Lyon, France

[$] The first two authors contributed equally to this work and share first authorship
[£] The last two authors contributed equally to this work and share last authorship

Article under finalization - to be submitted to *Environmental Health Perspective*

III.4.1 Introduction

There is growing evidence suggesting an association between exposure to pesticides and several diseases in humans (Alavanja and Bonner 2012; Bretveld et al. 2007; Damgaard et al. 2006; Noyce et al. 2012). However, reliably assessing environmental exposures to pesticides in epidemiological studies remains challenging, especially retrospectively. The use of geographic information systems (GIS) has been suggested as an efficient approach to improve the characterization of environmental pesticides exposures for households near agricultural areas (Nuckols et al. 2004; Zou et al. 2009). Previous studies have demonstrated that crop acreage proximate to households was a significant predictor of pesticides concentration in indoor dust (Gunier et al. 2011; Ward et al. 2006). Indoor dust is a repository of various chemicals, including pesticides. Protected from degradation by sunlight, fungus, and other factors, pesticides in indoor dust are more stable over time than in outdoor environs, and indoor dust sampling is considered as an efficient method to measure households' pesticide contaminations (Butte and Heinzow 2002;Lioy et al. 2002).

Crop acreage within a defined buffer were currently used to estimate environmental exposure to agricultural pesticides, but no consensus exists and buffer size vary depending on studies, e.g. 500m (Cockburn et al. 2011), 750m (Ward et al. 2006), 1000m (Carozza et al. 2009) or 1250m (Gunier et al. 2011). Also, only few studies included data on prevailing winds to improve the precision of GIS models (Brody et al. 2002; Brody et al. 2004; Chevrier et al. 2014; Pfleeger et al. 2006). It is likely that additional environmental parameters may influence pesticides drift from nearby agricultural fields to households, e.g. vegetative or structural barriers (De Schampheleire et al. 2009; Lazzaro et al. 2008; Ucar and Hall 2001). However, except the study from Brody et al. (2002) taking into account the presence/absence of forest, the impact of barriers has never been implemented in GIS models aiming to assess agricultural pesticide exposures. To our knowledge, the impact of prevailing winds and barriers on indoor agricultural pesticide contamination has not been assessed so far, and no consensus exists regarding the best buffer size to consider. Moreover, in the available studies, no distinction on the

crop type was made in the GIS metrics, despite of potential differences in terms of pesticide application materials and environmental settings (Ucar et al. 2001).

Based on measurement of pesticides in indoor dust, our study aimed to identify environmental determinants of the agricultural pesticide contamination in households close to typical French crops grown, in the Rhône-Alpes region, France. We also assessed the impact of the crop type on the pesticide drift from agricultural areas.

III.4.2 Methods

a) Study population

Our cross-sectional study was conducted in the Rhône-Alpes region, France, in 2012. We selected 645 households located in agricultural areas, less than 1000 meters from peach and apricot orchards (Zone 1), corn and grain cereal fields (Zone 2), and vineyards (Zone 3). Location and crop types were identified through the 2006 CORINE Land Cover® database (http://sd1878-2.sivit.org/) and data provided by the Departmental Agricultural Chambers (DAC). Overall, 612 eligible households were contacted by phone by using reverse directory, and 33 volunteered to participate spontaneously. After exclusion of 442 households declining participation or excluded due to occupational pesticide use of household members, 203 households were included in this study (69 in Zone 1, 66 in Zone 2, 68 in Zone 3). All participants signed consent forms. The study was approved by relevant French authorities (French National Commission of Informatics and Freedom, CNIL – n°1560501v0).

b) Data collection

Households were visited twice in 2012, spaced 30 days, during the predominant period of agricultural pesticide application on targeted crops (Zone 1: April-May; Zone 2: April-

June; Zone 3: June-July) according to DACs and previous air-quality measurements (ATMO Drôme-Ardeche 2010). During the first visit, a trained investigator (RB, JB) collected consent forms, measured global positioning systems (GPS) coordinates using a Tomtom® XL GPS receiver (TomTom NV, The Netherland), and administered a standardized questionnaire to collect household characteristics, including number of inhabitants, floor level, presence of pets, and domestic pesticide uses for pets, outdoor gardens, indoor plants, insects, and woodwork/framework during the two previous years. Whenever possible, missing responses were completed during the second visit.

At the second visit, we collected in each households two "recent dust" samples (RDS), one passive (dust trap) and one active (floor wipe), and one "old dust" sample (ODS; upper ledge of door- or window-frame wipe). For each samples, we recorded location (room) and height of collection points.

Sampling devices, sampling strategies and laboratory methods were described elsewhere (see part III.3 and Cettier et al. 2014). Briefly, dust traps were pure propylene wipe (28 x 28cm, Kimtech pure W4 ref. 7646, Kimberly-Clark® professional, UK) fixed on untreated wood frame using iron pins, and were left for 30 days near the main entrance at the first visit. Floor wipes were cellulose wipes (Kimtech science ref. 7552, 11cm x 21cm, Kimberly-Clark® professional, UK) moistened with 10mL of isopropanol. For each sample, 2 wipes were used to sample 1m², close to the main entrance door or in a cleared area in the kitchen or living-room. The homeowner was asked to not clean this area 7 days before the sampling. For ODS, we also used two cellulose wipes moistened with 10mL of isopropanol to sample upper door or window frame in the living room, entranceway or kitchen where dust had accumulated for at least 6 months.

Cellulose wipes were purified using dichloromethane and stored in similarly decontaminated glass boxes for transport to/from study homes. All samples were placed in separate, clean, decontaminated, stoppered Pyrex flasks, stored in a cooler at ambient temperature immediately after sampling (icetime® 26 liters, Campingaz, France). Samples were transported by car within 3 days to the laboratory for analysis. At the end

of each sampling period, 2 cellulose wipes remaining in the glass storage container used for transport were moistened with 10mL isopropanol, placed in a flask, and transported to the lab to serve as blanks for quality control of the sampling procedure. The same procedure (without isopropanol) was followed for polypropylene wipes (dust trap).

We employed a method similar to that described by Bernard et al. (2008) to extract and measure the mass of 406 organic pesticides, 10 pesticide metabolites, and piperonyl butoxide in the collected samples (see part III.3). Briefly, we extracted compounds by adding 150 mL of dichloromethane in each flask, which was agitated 4 hours. The solution was filtrated and concentrated to 1mL under nitrogen steam. The solution was separated in two equal parts and conditioned to be analyzed by gas chromatography coupled with mass spectrometer and by high performance liquid chromatography coupled with tandem of mass spectrometer. For all samples, extractions of internal standards (Chrysene D12, hexabromobenzene, and triphenylphosphate) were within 20 % around the expected concentration. The limit of detection by both methods was 1ng/mL for all compounds. Analyses were performed in accordance with international quality standards (ISO-17025, http://www.iso.org/). No contamination was found in blanks, except orthophenyphenol in two dust trap blanks performed in Zone 3, at concentrations similar to median. Efficiency and repeatability of the cellulose wipe were assessed elsewhere (Cettier et al. 2014).

c) Geographical information system

Using ArcMap 10.0 (ArcGIS desktop, ESRI, Redlands, CA), households were geolocated in the GIS based on their GPS coordinates (converted in Lambert 93). The land use coverage was set within 1,250 m for all study households, using spatially registered land-cover data for vineyards ("*Casier Viticole Automatisé*" database; Directorate-General of Customs and Indirect Taxes (DGDDI)) and other crop types ("*Registre Parcellaire Graphique*" database (RPG); Regional Directorate for Food, Agriculture and Forestry (DRAAF)). All land-cover data were from 2012, and crop locations and types were field

verified by EF, RB and JB. We calculated the total acreage of targeted crops (orchards and cereals in Zone 1; cereals in Zone 2; vineyards in Zone 3) for different buffer sizes (250, 500, 750, 1,000, and 1,250m) around each household.

For each buffer, we defined 8 contributive areas for pesticide drift (CAP), based on cardinal directions: North; Northeast; East; Southeast; South; Southwest; West; and Northeast (Figure 3.7). Each household was assigned to a meteorological station based on proximity and topographical considerations. Day per day data on prevailing wind directions were provided by *Météo* France. We calculated the proportion of wind blowing for the eight cardinal directions, corresponding to the eight CAP, over the sampling period (between the first and the second visit). To account for wind effect in the GIS, we created a variable called "*effective contributing area for pesticide drifts"* (ECA), which was estimated for each target crops, for each household, separately for each buffer size (250–1250m). For a given buffer, ECA corresponds to the sum of the targeted crop acreage in each CAP weighted by the corresponding proportion of prevailing wind direction:

$$\mathbf{ECA}_{\mathbf{household}} = \sum(\mathbf{crop\ acreage}_{\mathbf{CAP}}\ (\mathbf{m^2}) \times (\mathbf{Wind\ direction}_{\mathbf{CAP}}\ (\%)))$$

To account for the influence of barriers onto pesticides drift, we focused on three different types of barriers: vegetative barriers (VB), structural barriers (SB), such as buildings, and topographic barriers (TB) (Figure 3.7). To define VB, presence of vegetation in 2012 was determined using the vegetation theme of a national land-use database (*BD topo*®; 1/10,000; French National Geographic institute (IGN)). To define SB, presence of buildings in 2012 was determined using *BD topo*® and *BD parcellaire*® (1/2,000; IGN), a national database of infrastructures. To define TB, we used a national elevation database (*BD alti*®; 25m scale; IGN) to determine all areas located at higher elevation than households. *BD topo*® and *BD parcellaire*® provide information on the land uses and infrastructures, while *BD alti*® provide level curve information (scale: 1/10,000, 1/2,000, and 25m, respectively). Only barriers crossing a CAP from side to side and situated between the household and a section of the targeted crop within this CAP

have been considered. For each CAP and for each type of barriers, the score ranged from 0 (absence of barrier) to 1 (at least one barrier). The maximal score for each buffer and each type of barrier was eight. Crop acreage, ECA, and barriers were determined for each household and buffer size using an automated process based on ArcGIS models builder (ESRI, Redlands, CA).

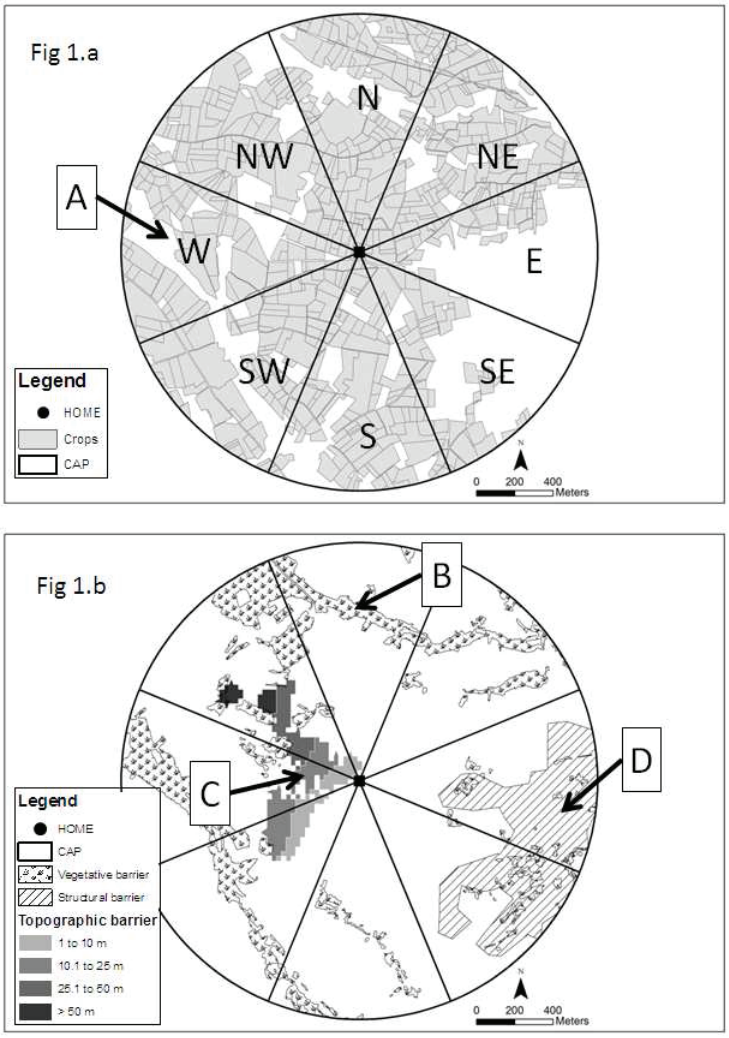


Fig. 1.a*: Eight contributive areas for pesticide drift (CAP) have been defined for each household, one for each cardinal direction (A represent the west CAP).* ***Fig. 1.b****: Presence of vegetation (B), relief (C), and buildings (D) were represented on each CAP. Only barriers crossing a CAP from side to side and situated between the household and a targeted crop area within this CAP, were taken into account (e.g. B and C). .*

Figure 3.7: Representation of contributive areas for pesticide drift and potential related barriers

d) Pesticide selection for data analysis

Overall, 156 distinct pesticides were detected in dust samples (see part III.3). For the present study, we selected 64 pesticides authorized on orchards, cereals, and vineyards, based on information from the registry of the French Agricultural Ministry (e-phy; http://e-phy.agriculture.gouv.fr/) and the European Union Pesticide database (http://ec.europa.eu/sanco_pesticides/public/index.cfm). Only compounds detected in at least 10% of households of each zone were retained for analyses (Table 3.6). Considering RDS (floor wipes and dust trap samples combined), analyses were made on four, two, and 11 pesticides for Zone 1 (orchards and cereals), Zone 2 (cereals), and Zone 3 (vineyards), respectively (detection rate: 10 – 56%). Among these, two, one, and six pesticides were retained for analyses on dust traps (detection rate: 10 – 41%; median quantity per sample: 26 – 10,755 ng), and three, two and 11 pesticides were retained for analyses on floor wipes (detection rate: 10 – 54%; median quantity per sample: 8 – 1,350 ng). Considering ODS, we kept 11, five, and 16 compounds for analyses on Zone1 (only nine for cereals), 2 and 3, respectively (detection rate: 10 – 88%).

Table 3.6: Detection rate and quantity of pesticides retained for statistical analyses

	Recent Dust Samples					Old dust samples
	Overall detection in recent dust samples[a]: n (%)	Detection in dust trap: n (%)	Amount in dust traps samples (median ng + IQR[b]) – 30 days	Detection in floor wipe: n (%)	Amount in floor wipe samples (median ng + IQR[b]) – 7 days	Detection in old dust samples: n (%)
Orchards / Cereals – Zone 1 (69 households)						
Bifentrine[c]	*<10%*		–		–	**10 (14%)**
Chlorpyriphos[c]	**20 (29%)**	**7 (10%)**	855 (295 – 1,095)	**20 (29%)**	66 (38 – 101)	**43(62%)**
Cyproconazole	*<10%*		–		–	**10 (14%)**
Cyprodinil[c]	*<10%*		–		–	**17 (25%)**
Fenbuconazole[cd]	*<10%*		–		–	**15 (22%)**
Fludioxinil[c]	*<10%*		–		–	**19 (28%)**
Imidacloprid[c]	**8 (12%)**	*<10%*	–	*<10%*	–	**9 (13%)**
Oryzalin[cd]	*<10%*		–		–	**39 (57%)**
Piperonyl butoxide[c]	**19 (28%)**	**10 (14%)**	333 (127 – 648)	**17 (25%)**	124 (60 – 560)	**21 (30%)**
Tebuconazole[c]	**18 (26%)**	*<10%*	–	**18 (26%)**	10 (7 – 14)	**52 (75%)**
Thiacloprid[c]	*<10%*		–		–	**7 (10%)**
Cereals – Zone 2 (66 households)						
Acetochlor	*<10%*		–		–	**14 (21%)**
Metolachlor	*<10%*		–		–	**9 (14%)**
Piperonyl butoxide[c]	**23 (35%)**	**17 (26%)**	85 (45 – 465)	**17 (26%)**	91 (59 – 315)	**20 (30%)**
Propiconazole[c]	**15 (23%)**	*<10%*	–	**14 (21%)**	14 (7 – 16)	**29 (44%)**
Tebuconazole[c]	*<10%*		–		–	**26 (39%)**
Vineyards – Zone 3 (68 households)						
Azoxystrobin[c]	*<10%*		–		–	**8 (12%)**
Boscalid	*<10%*		–		–	**8 (12%)**
Chlorpyriphos[c]	**7 (10%)**	*<10%*	–	**7 (10%)**	16 (13 – 38)	**11 (16%)**
Chlorpyriphos methyl	**13 (19%)**	**13 (19%)**	380 (120 – 650)	**8 (12%)**	27 (15 – 55)	*<10%*
Cymoxanil[c]	**33 (49%)**	**28 (41%)**	21 (10 – 37)	**25 (37%)**	12 (7 – 20)	*<10%*
Cyprodinil[c]	**15 (22%)**	**10 (15%)**	79 (54 – 183)	**12 (18%)**	17 (11 – 20)	**14 (21%)**
Fluazinam	**32 (47%)**	**26 (38%)**	184 (119 – 290)	**21 (31%)**	9 (5 – 15)	**15 (22%)**
Fludioxinil[c]	*<10%*		–		–	**10 (15%)**
Flufenoxuron	*<10%*		–		–	**17 (25%)**
Folpet	**23 (34%)**	**22 (32%)**	10,755 (5,916 – 17223)	**9 (13%)**	1,350 (430 – 4,450)	**60 (88%)**

Kresomix methyl	**10 (15%)**	*<10%*	–	**7 (10%)**	79 (52 – 93)	**7 (10%)**
Iprovalicarb	*<10%*		–		–	**12 (18%)**
Oryzalin[c]	*<10%*		–		–	**15 (22%)**
Pyrimethanil[c]	**13 (19%)**	**9 (13%)**	26 (20 – 41)	**8 (12%)**	8 (6 – 15)	**7 (10%)**
Spiroxamine	**28 (41%)**	*<10%*	–	**28 (41%)**	12 (7 – 20)	**14 (21%)**
Tebuconazole[c]	**38 (56%)**	*<10%*	–	**37 (54%)**	12 (7 – 20)	**37 (54%)**
Trifloxistrobin[c]	**11 (16%)**	*<10%*	–	**8 (12%)**	74 (54 – 133)	**8 (12%)**
Zoxamide	*<10%*		–		–	**10 (15%)**

[a]:detection either on dust trap or floor wipe samples; [b]: interquartile range; [c]: agricultural pesticides also used for domestic purposes; [d]: authorized for orchards but not for cereals. This table presents pesticides found in recent dust and old dust samples authorized for orchards and cereals, cereals, and vineyards in 2012, and detected in at least 10% of recent or old dust samples from households in Zone 1, 2, and 3, respectively.

e) Data analyses

RDS data were expressed as: (i) presence-absence of compounds in households (either from home's dust trap or floor wipe samples) (ii) quantity of compounds in dust trap and floor wipe samples separately, in ng (log+ 1 transformed). Because of limitations in standardizing the sampling surface and the period of dust accumulation, only presence-absence of compounds were considered for ODS.

All analyses were performed separately for each of the three zones. Given the important acreage of cereals (corn and grain) crops around homes in zone 1, we run separate analyses for orchards and cereals in Zone 1. Given the number of pesticides and their low detection rates (<30% for most of them), we used multivariate statistical methods (meaning here simultaneous analysis of more than one dependent variable). For pesticides quantities, we used Redundancy Analysis (RDA), a multi-response analogue of linear regression, combining regression and Principal Component Analysis (PCA) (Borcard et al. 2011). It enables variability partitioning and testing of statistical significance of the relationship(s) between the response matrix and explanatory variable(s) (p-values assessed using permutation tests). For pesticides presence-absence, we used distance-based RDA (db-RDA) which provides similar information than RDA

but can be adapted to qualitative data (Legendre and Anderson, 1999), the distance between households being here the simple matching coefficient (number of presence-presence or absence-absence matches over the number of compounds). We performed Tobit (single-pesticide) quantitative analyses for pesticides detected in at least 30% of RDS (dust trap and floor wipe samples separately). According to Lubin et al. (2004) using Tobit regressions may induced biased variance when 30% or more data are below the detection limits. Overall, only 5 pesticides used on vineyards in Zone 3 met this criterion (Table 3.6).

By db-RDA, we first defined the variability of pesticide presence-absence in RDS explained by crop acreage within different buffer sizes (250m – 1,250m). For each crop type, the optimal buffer size was determined as the one for which the crop acreage explained most variability. In a second step, for the optimal buffer size for each crop type, we examined the following potential determinants: crop acreage (log+1 transformed), ECA (log+1 transformed), number of vegetative/topographic/structural barriers, presence of pets, number of inhabitants, domestic use of pesticides within the past two years for pet treatment, indoor treatment, and outdoor treatment. Using the above-mentioned statistical methods, we evaluated the significance of each potential determinant one at a time (univariate models). Then, we created final, multivariate models following a forward procedure to select variables with p-value < 0.1 to include in the final models.

To check for consistency, we performed db-RDA of pesticide presence-absence in ODS. Further we performed sensitivity analyses, by repeating db-RDA and RDA analyses in RDS excluding potential clusters of households (households located within 50m from each other: seven, nine, and four in Zone 1, 2, and 3, respectively). Only the first household sampled was kept to constitute a cluster-free data set. All statistics were performed with R 3.0.0, using packages *vegan* (Oksanen et al., 2013) for RDA and db-RDA, and *censReg* (Henningsen, 2013) for Tobit regression.

III.4.3 Results

a) Households' characteristics and agricultural pesticide contamination

Table 3.7 presents characteristics of the 203 study households. Median number of household members was similar across zones (2 – 3). Households were mainly single level dwelling (99%). Sixty-one percent had pets, 41% used pesticides to treat pets, 52% used pesticides outdoor, and 77% used pesticides indoor. All crops combined, agricultural fields within the 1000m buffer covered 46,8%, 39,5%, and 53,2% of the total area (median values) for zones 1, 2, and 3, respectively. Median acreage of targeted crop varied from 0 (250m buffer size) to 17.5 ha (1,250m buffer size) for orchards and from 4 to 134.2 ha for cereals in Zone 1; from 0.9 to 130.9 ha for cereals in Zone 2; and from 5 to 135.3 ha for vineyards (Zone 3). Median ECA varied from 0.1 (orchards) to 2.5 (vineyards) for the 500m buffer size, and from 2.2 (orchards) to 12.3 (cereals in Zone 1) for the 1000m buffer size. For the 500m buffer size, the median barrier scores varied from 0 to 1 (topographic and structural barriers), or was null (vegetative barriers). For the 1,000m buffer size, the median barrier scores varied from 1 to 2 (vegetative), from 0 to 3 (topographic), and from 0 to 6 (structural barriers).

Table 3.7: Household characteristics

	Zone 1 (n=69)	**Zone 2 (n=66)**	**Zone 3 (n=68)**
Inhabitant per households (median, IQR)	2 (2 – 4)	3 (2 – 4)	2 (2 – 4)
Houshold at ground level: n (%)	67 (97%)	66 (100%)	67 (99%)
Pets: n (%)	44 (64%)	38 (58%)	42 (62%)
Pesticides usage for pets: n (%)	26 (59%)	27 (71%)	31 (74%)
Outdoor domestic usage: n (%)	29 (45%)	33 (60%)	43 (67%)
Indoor uses of pesticides[a]: n (%)	54 (78%)	53 (80%)	50 (74%)
Vegetative barriers (median, IQR)			
500m buffer size	0 (0 – 1)	0 (0 – 2)	0 (0 – 1)
1000m buffer size	1 (0 – 2)	1 (0 – 2)	2 (0 – 3)
Topographic barriers (median, IQR)			
500m buffer size	0 (0 – 0)	0 (0 – 0)	1 (0 – 4)
1000m buffer size	0 (0 – 0)	0 (0 – 0)	3 (1 – 5)
Structural barriers (median, IQR)			
500m buffer size	0 (0 – 0)	1 (0 – 4)	0 (0 – 1)
1000m buffer size	0 (0 – 2)	6 (1 – 8)	1 (0 – 6)

Acreage of targeted crops[b]: ha (median, IQR)	**Orchards (Zone 1)**	**Cereals (Zone1)**	**Cereals (Zone2)**	**Vineyards (Zone 3)**
250m buffer size	0 (0 – 2.2)	4 (0.8 – 8.2)	0.9 (0 – 4.1)	5 (0 – 8.5)
500m buffer size	2.5 (0 – 10.4)	18.9 (9.3 – 31.2)	9.9 (4.6 – 22.8)	23.5 (0.2 – 39.1)
750m buffer size	9.7 (1.5 – 20.2)	43.2 (30.4 – 69)	34.4 (25.2 – 59.3)	53.3 (5.0 – 89.3)
1000m buffer size	13.4 (6.4 – 33.2)	86.1 (58 – 125)	77.9 (61 – 112)	93.8 (19.8 – 153.5)
1250m buffer size	17.5 (12.7 – 51.1)	134.2 (84.8 – 203.6)	130.9 (115.2 – 174.8)	135.3 (49.2 – 239.3)
ECA (median, IQR)	**Orchards (Zone 1)**	**Cereals (Zone1)**	**Cereals (Zone2)**	**Vineyards (Zone 3)**
500m buffer size	0.1 (0 – 1.2)	1.5 (0.4 – 3.5)	1.3 (0.5 – 3)	2.5 (0 – 4.8)
1000m buffer size	2.2 (0 – 3.9)	12.3 (6 – 18.9)	10.2 (9 – 14.7)	11 (1.7 – 18.6)

IQR : Inter-quartile range; ECA :effective contributing area for pesticide drifts.
[a]: Either pets, garden, plants, or indoor treatments (flying, crawling or xylophage bugs; fungus)
[b]: Other crops observed are detailed in Supplemental Materials (Table S7) for the 1000m buffer size

b) Optimal buffer size according to crop types

Based on presence-absence of agricultural pesticides in RDS, Table 3.8 presents the variability of pesticide presence-absence explained by the crop acreage, for different buffer sizes (250m–1,250m).The optimal buffer size was 500m for orchards (Zone 1) and for cereals in Zone 2, and 1,000m for cereals in Zone 1 and for vineyards (Zone 3), with 3.5% (p=0.049), 3.5% (p=0.1), 5.4 (p=0.005) and 10.9% (p=0.005) of variability explained, respectively. These buffer sizes were used for subsequent analyses.

Table 3.8: Variability of the agricultural pesticide contamination explained by the crop acreage for different buffer sizes

Buffer sizes	*Orchards (Zone 1)*		*Cereals (Zone 1)*		*Cereals (Zone 2)*		*Vineyards (Zone 3)*	
	%var	p-value	%var	p-value	%var	p-value	%var	p-value
250m	2	0.26	3.2	0.07	0.3	0.85	9.0	0.005*
500m	**3.5**	**0.049***	4.1	0.034	**3.5**	**0.13**	6.8	0.005*
750m	2.6	0.1	2.1	0.3	2	0.31	9.1	0.005*
1000m	1.8	0.29	**5.4**	**0.005***	0.9	0.42	**10.9**	**0.005***
1250m	2.5	0.18	3.7	0.027	3.5	0.12	8.6	0.005*

%var: proportion of variability of the exposure to agricultural pesticides explained by the model. Results were obtained using db-RDA. P-values were estimated by permutation tests. Analyses were made on qualitative data from recent dust samples: pesticides were considered as present in the households if detected either on dust trap or floor wipe samples.

c) Determinants of the contamination of recent dust samples (univariate models)

For the selected buffers, univariate models identified the following factors as significant determinants of agricultural pesticides presence-absence (qualitative data; Supplemental Materials, Table S1): crop acreage (p=0.049) and indoor domestic use of pesticide (p=0.025) for orchards; crop acreage (p=0.005) and indoor domestic treatment (p=0.025) for cereals in Zone 1; topographic and structural barriers (p=0.01 and p=0.44, respectively) for cereals in Zone 2; and crop acreage (p=0.005) and ECA (p=0.005) for vineyards.

Based on agricultural pesticide concentration in dust trap samples (quantitative data), univariate models identified crop acreage (vineyards; p=0.005) and ECA (orchards, p=0.01; vineyards, p=0.005) as significant determinant of the indoor dust contamination (Supplemental Materials, Table S2). RDA could not be performed in Zone 2 since only one agricultural pesticide was detected in more than 10% of households. Considering agricultural pesticide concentrations in floor wipe samples, we found that crop acreage (cereals (Zone1), p=0.01; vineyards, p=0.005), ECA (vineyards, p=0.01), structural barriers (cereals (Zone 2), p=0.043), and indoor domestic treatment (cereals (Zone1),

p=0.013) were significant determinants of the agricultural pesticide contamination (Supplemental Materials, Table S3).

d) Determinants of the contamination of recent dust samples (multivariate models)

Table 3.9 presents the variability of agricultural pesticide contamination explained by multivariate models in RDS. Variability explained by the different models was 7.1 – 8.3% for orchards; 3.7 – 12.3% for cereals in Zone1; 8.5 – 9.5% for cereals in Zone 2; 14.2 – 18.3% for vineyards. Variables included in stepwise models were indoor domestic treatments, acreage, and ECA for orchards; acreage, indoor domestic treatment, and ECA for cereals in Zone 1; topographic and structural barriers for cereals in Zone 2; acreage, ECA, and vegetative barriers for vineyards.

Table 3.9: Variability of the exposure explained using complete multivariate models in recent dust samples

	Models	Variable included in the models	Variability explained (%)
Orchards in Zone 1 (500m)	A	indoor domestic treatments + acreage	7.1
	B	acreage + ECA	9.2
	C	indoor domestic treatments + ECA	8.3
Cereals in Zone 1 (1000m)	A	indoor domestic treatment + acreage + ECA	12.3
	B	acreage	3.7
	C	indoor domestic treatment + acreage	11.4
Cereals in Zone 2 (500m)	A	topographic barrier + structural barrier	9.5
	B	–[a]	–[a]
	C	topographic barrier + structural barrier	8.5
Vineyards in Zone 3 (1000m)	A	acreage + vegetative barrier	15.2
	B	acreage + vegetative barrier	18.3
	C	acreage + ECA + vegetative barrier	14.2

ECA: "effective contributing area for pesticide drifts".
[a] analysis was not possible because only one agricultural compound was above 10% of detection.
Completes models are based on stepwise procedure. Models A were based on multivariate analyses of qualitative data (detection of pesticides either in dust trap of floor wipes) using db-RDA. Models B were based on quantitative results for dust trap samples using RDA. Models C were based on quantitative results from floor wipes samples using RDA.

Pesticides detected in more than 30% of households of Zone 3 (dust trap and floor wipe samples separately) were analyzed individually using Tobit regressions. Factors considered as determinants of the pesticides concentration in the multivariable, stepwise regression model (threshold p=0.1) were acreage, vegetative barrier, ECA and pet treatments (Table 3.10). Variability explained by multivariate models depending on the compounds analyzed varied from 1.5 (fluazinam) to 20.1% (cymoxanil) in dust trap samples, and from 2.2 (fluazinam) to 15% (cymoxanil) in floor wipe samples.

Table 3.10: Tobit regression models on recent dust for pesticides having detection rate >30%

	Compounds	Variable included in the models	Variability explained (%)
Dust trap samples	Cymoxanil	acreage + vegetative barrier + pet treatments	20.1
	Fluazinam	acreage	1.5
	Folpet	acreage + vegetative barrier	9.9
Floor wipe samples	Cymoxanil	acreage + vegetative barrier	15.0
	Fluazinam	acreage + ECA + vegetative barrier	6.6
	Spiroxamine	acreage	2.2
	Tebuconazole	acreage	3.6

ECA: "effective contributing area for pesticide drifts".
Stepwise multivariate models were based on quantitative data of pesticides detected in RDS of households from Zone 3 having at least 30% of detection rate (no compounds reach this level in Zone 1 and 2). Variables were defined for the 1000m buffer size.

e) Old dust samples and sensitivity analyses

Based on presence-absence of agricultural pesticides in ODS of study households, we found that the buffer explaining the most of the variability was 500m for orchards (12.7%, p=0.005), 1,000m for cereals in Zone 1 and 2 (6%, p=0.01 and 3%, p=0.14, respectively), and 1,000m for vineyards (10.6%, p=0.005) (see Supplemental Material, table S4). Based on these buffer sizes, Table 3.11 presents the variability of the agricultural pesticide contamination explained by multivariate models in ODS (results of univariate analyses are presented in Supplemental Materials (table S5)). Variability

explained by the models varied from 9.2% (cereals in Zone 2) to 14.0% (vineyards), to 17.6% (orchards), and to 17.6% (cereals in Zone 1). Factors identified in stepwise models were indoor domestic treatments and ECA for orchards; indoor domestic treatments, acreage, vegetative barriers, and structural barriers for cereals in Zone 1; indoor domestic treatments, acreage, and vegetative barriers for cereals in Zone 2; indoor domestic treatments and acreage for vineyards.

Excluding potential clusters of households from multivariate analyses in RDS showed only small changes in the variability explained by the models (Supplemental Material, Table S6, to be compared to Table 3.9).

Table 3.11: Variability of the exposure explained using complete multivariate models in old dust samples

	Variable included in the forward models	Variability explained (%)
Orchards in Zone 1 (500m)	Indoor domestic treatments + ECA	17.6
Cereals in Zone 1 (1000m)	Indoor domestic treatments + acreage + vegetative barriers + structural barriers	17.6
Cereals in Zone 2 (1000m)	Indoor domestic treatments + acreage + vegetative barriers	9.2
Vineyards in Zone 3 (1000m)	Indoor domestic treatments + acreage	14.0

ECA: "effective contributing area for pesticide drifts".
Model based on db-RDA from ODS qualitative data (presence-absence of pesticides).

III.4.4 Discussion

To our knowledge, this is the first study simultaneously assessing the impact of prevailing winds and physical barriers (vegetative, topographic, and structural) on agricultural pesticides contamination in indoor dust. Moreover, our study suggested crop specific distances of pesticides drift from agricultural areas in the Rhône-Alpes region (France). Another unique feature of our study was to assess pesticides contamination in

recent dust as well as cumulative pesticide exposure (old dust) to identify environmental determinants of both recent and past indoor agricultural pesticides contamination.

Our results confirmed previous findings suggesting crop acreage proximate to households as a significant determinant of the indoor agricultural pesticides concentration (Gunier et al. 2011; Ward et al. 2006). Gunier et al. (2011) showed that crop acreage around study homes was significantly correlated with pesticide concentration in indoor carpet dust, for five of seven pesticides investigated (including chlorpyriphos, also screened in our study, but not individually). The authors found stronger correlations with crop acreage when using a 1,250m buffer size compared to a 500m buffer size. Ward et al. (2006) suggested that corn and soybean fields acreage within 750m from households was a significant predictor of the herbicide level in carpet dust, but they recommended further studies to assess the impact of crop acreage within buffers larger than 750m. These findings were overall consistent with our results concerning cereals in Zone 1 and vineyards (best results for 1000m buffer sizes), but not for orchards and cereals in Zone 2 (500m buffer size). However, no specific analyses have been conducted on orchards in these studies, and differences in terms of the landscape characteristics and agricultural spraying practices would exist between crop types (Ucar et al. 2001). Regarding cereals, the best buffer size based on RDS was 500m in Zone 2, but 1000m in Zone 1. However, results in Zone 2 should be considered with caveats since the relationship was not statistically significant, and only two agricultural pesticides were included in our analyses (propiconazole and piperonil butoxide; both declared to be used for domestic purpose by study participants). Since analyses of cereals in Zone 1 in RDS were consistent with analyses in ODS (zone 1 and 2) and based on more agricultural pesticides, we consider that the best buffer size for cereals would be 1000m.

The overall consistency of our multivariate models between zones and dust types strengthens our conclusions concerning the potential determinants of the environmental exposure to agricultural pesticides (Tables 3.9–3.11). However, the different multivariate models explained only a modest proportion of the variability in the pesticides contamination in indoor dust (7.1 – 18.2%), and we observed strong difference in

variability explained when focusing on specific pesticides (1.5 – 20.1%). In comparison, Gunier et al. (2011) explained between 4 and 28% of the variability of the pesticide exposure, depending on the metric and the pesticides considered. Similarly, GIS models developed by Withehead et al. (2011) only explained 15% of the variation in polycyclic aromatic hydrocarbons in house dust. These results suggested that additional variables should impact the pesticide drift and that misclassification may occur for specific pesticides when assessing exposure using GIS.

We found that prevailing winds and vegetative barriers were identified as significant determinants of the agricultural pesticides presence and concentration in the indoor dust, suggesting their influence on the pesticides drift from agricultural areas. This confirms previous findings by Chevrier et al. (2014) suggesting more significant correlation between presence of crops and concentration of metolachlor in urine of pregnant women when the major wind direction was incorporated in the regression model ($p=0.03$ vs $p=0.001$). Previous methodological studies also suggested that physical barriers limit pesticide drifts (De Schampheleire et al. 2009; Lazzaro et al. 2008; Ucar and Hall 2001). Since crop acreage and ECA have been identified simultaneously in several multivariate models using the stepwise modeling approach, it appears that ECA adds information to acreage alone and significantly improved our models.

Indoor domestic use of pesticides has also been identified as determinant of the exposure in Zone 1 (RDS) and in ODS (all zones). It is not surprising since some agricultural pesticides were declared to be used in the domestic context. Indoor domestic use had stronger impact than outdoor domestic use on the indoor dust contamination, but our analyses were limited to pesticides used in agriculture. In a previous publication, we have already shown that domestic usage is an important source of indoor dust contamination (see part III.3). Information on indoor pesticide use should therefore be collected in future studies, especially when focusing on pesticides having mixed agricultural and domestic usage.

In our study, presence of pets was not associated with pesticides contamination in indoor dust, despite of that having a dog has been previously associated with higher concentrations of chlorpyriphos in repeated carpet dust samples from 21 Californian households (Deziel et al. 2013). It is difficult to explain this difference. However, our study was based on a larger number of households and our findings were consistent across the different zones and models. Similarly, pet treatment was not identified as determinant of contamination, but the pesticide reported to be the most frequently used for pet treatment (fipronil), was banned in 2005 for agricultural use in France, and thus, not retained for our analyses.

Models explained higher variability of the exposure based on RDS in Zone 3, compared to Zone 1 and 2. These findings may be explained by the higher number of agricultural pesticides retained in analyses, and the overall high crop acreage. Concerning Zone 1, households are exposed to both orchards and cereals, and pesticides retained in analyses are authorized on both crop types (except two in ODS). Thus, each crop type explained only a part of the overall variability explained the overall agricultural areas. By taking both cereals and orchards into account, the overall variability explained would be closer to results observed in Zone 3. In Zone 2, the low variability explained would be explained by the low agricultural pesticide detection rates. It is however difficult to interpret if it was related to lower agricultural pesticide contaminations or difficulties to collect and measure pesticide contaminations in this area.

ODS allowed collection of higher dust quantities accumulated during longer time periods, explaining increased detection rate, while pesticides exposures could have been missed by the RDS due to short time periods of dust collections. However, since pesticides are accumulated in settled dust, it is not possible to distinguish whether pesticide contaminations in ODS corresponds to current or past usage of pesticides. Lower explained variability in pesticide presence-absence were observed in Zone 2 and 3 when using ODS compared to RDS, but it was the opposite effect for Zone 1 (orchards and cereals), probably due to a larger number of pesticide included in analyses of ODS for

this area. Combining RDS and ODS is an interesting approach to assess environmental pesticides contamination and should be considered in future studies.

Dust trap and floor wipes targeted different pesticide profiles, probably due to differences regarding the location of the sampling area (Edwards et al. 1998) or differences in terms of physic-chemical properties (Cettier et al. 2014). By merging qualitative data from dust traps and floor wipe samples, we obtained a broader overview in terms of pesticides contamination. However, since we were not able to assess the total amount of each pesticide applied on each crop proximal to study households and since the sampling procedure differed between these two approaches, we chose not to merge quantitative data from dust trap and floor wipe samples.

Our study had several limitations that might have reduced the proportion of variability explained by our models. First, since France does not a have pesticide use registry, we did not know exactly which pesticides were applied when, where and at which concentrations. Second, low quantities of dust sampled by our sampling strategy probably explain some of the measures below the detection limits and low detection rate, due to analytical limitations. This might have impacted the overall variability explained by our different models. Third, crop growing in France is fragmented and diverse, and crop types other than those targeted by us are present in the different sampling areas. It might have induced potential contaminations since agricultural pesticides used in our targeted crops might have been used in the other crop type of the area.

Ucar and Hall (2001) showed variations in the wind velocity near natural or artificial barriers, which would impact on the air-born pesticide drift. However, these variations were observed for a short distance before and after the barrier. Further investigation should be conducted to assess the role of the barriers and the best manner to express these in the GIS (i.e by delimiting specific area of potential effect before and after the barrier). Since pesticide drift are likely depending on the environmental context and may differ from area to area, replication studies are needed to confirm our findings in other places and agricultural settings.

III.4.5 Conclusion

Our results suggest that crop acreage close to households, predominant winds, and presence of vegetative barriers are significant determinants of the indoor dust contamination by agricultural pesticides. Distance of pesticides drift should be influenced by the crop type, and best buffer sizes to consider were from 500m (orchards) to 1000m (cereals and vineyards). Our findings and the new promising approaches we developed to express wind and presence barriers based on "*contributive area for pesticide drift*" will serve as basis for more precise GIS metrics for assessing environmental exposure to agricultural pesticides.

Acknowledgment:
The authors acknowledge Kevin Saout (Master student, Centre Léon Bérard) for collecting part of the samples; all the volunteers who participated in this study. Rémi Béranger holds a doctoral grant from the Région Rhône-Alpes. This project was granted by the Fondation de France (Engt 2011-00023939) and the Rhône-Alpes Regional Council (ref. 12-021795-01).

III.4.6 Supplemental Materials

Environmental determinant of the indoor exposure to agricultural pesticides

Béranger R, Billoir E, Nuckols JR, Faure E, Blain J, Chasles V, Philip T, Schüz J, Fervers B

Table S1. Potential determinants of the agricultural pesticide exposure based on univariate analyzes of pesticide presence-absence in recent dust samples

	Orchards in Zone 1 (500m)		Cereals in Zone 2 (1000m)		Cereals in Zone 2 (500m)		Vineyards in Zone 3 (1000m)	
	%var	p-value	%var	p-value	%var	p-value	%var	p-value
Crop acreage	**3.5**	**0.049**	5.4	0.005	3.5	0.13	**10.9**	**0.005**
ECA	2.3	0.2	1.2	0.48	3.2	0.13	8.4	0.005
Vegetative barrier	1.5	0.41	0.7	0.69	0.4	0.78	**1.6**	**0.58**
Topographic barrier	1.5	0.43	2.7	0.13	**5.6**	**0.01**	1.5	0.39
Structural barrier	0.4	0.91	0.8	0.73	**4.6**	**0.044**	0.6	0.9
Presence of pet	2.8	0.11	2.8	0.11	1.3	0.45	0.9	0.7
Pet treatments	2	0.31	2	0.31	4.1	0.06	0.7	0.81
Outdoor domestic treatments	0.8	0.7	0.8	0.7	0.4	0.72	1	0.58
Indoor domestic treatments	**4.3**	**0.025**	**4.3**	**0.025**	2	0.21	0.6	0.89
Number of inhabitants	0.3	0.93	0.3	0.93	0.9	0.61	0.7	0.87

%var: percentage of variability of the exposure to agricultural pesticides explained by the variable; ECA: Effective Contributing Area for pesticide drifts. Results were obtained using distance-based redundancy analyses (db-RDA). A pesticide was considered as present in the households if detected either on dust trap or floor wipe samples. Variables retained in the multivariate models using forward stepwise approach (threshold p=0.01) are presented in bold.

Table S2. Potential determinants of the agricultural pesticide exposure based on univariate analyzes of quantitative measures in dust traps samples

	Orchards in Zone 1 (500m)		Cereals in Zone 2 (1000m)		Cereals in Zone 2 (500m)		Vineyards in Zone 3 (1000m)	
	%var	p-value	%var	p-value	%var	p-value	%var	p-value
Crop acreage	**3.5**	**0.079**	**3.7**	**0.07**	*NA*	*NA*	**12.3**	**0.005**
ECA	**5.7**	**0.01**	0.2	0.94	*NA*	*NA*	11.3	0.005
Vegetative barrier	0.4	0.82	0.9	0.51	*NA*	*NA*	**2.0**	**0.18**
Topographic barrier	1.2	0.29	1.6	0.36	*NA*	*NA*	2.6	0.11
Structural barrier	1.3	0.41	2.5	0.12	*NA*	*NA*	0.6	0.85
Presence of pet	3.6	0.078	3.6	0.078	*NA*	*NA*	1.2	0.45
Pet treatments	1.1	0.54	1.1	0.54	*NA*	*NA*	1.4	0.4
Outdoor domestic treatments	1.0	0.55	1.0	0.55	*NA*	*NA*	0.4	0.96
Indoor domestic treatments	2.7	0.105	2.7	0.105	*NA*	*NA*	0.9	0.67
Number of inhabitants	1.7	0.46	1.7	0.46	*NA*	*NA*	0.5	0.89

%var: percentage of variability of the exposure to agricultural pesticides explained by the variable; ECA: Effective Contributing Area for pesticide drifts. Results were obtained using redundancy analyses (RDA). P-value was estimate using permutation tests. Variables retained in the multivariate models using forward stepwise approach (threshold p=0.01) are presented in bold.

Table S3. Potential determinants of the agricultural pesticide exposure based on univariate analyzes of quantitative measures in floor wipes samples

	Orchards in Zone 1 (500m)		Cereals in Zone 2 (1000m)		Cereals in Zone 2 (500m)		Vineyards in Zone 3 (1000m)	
	%var	p-value	%var	p-value	%var	p-value	%var	p-value
Crop acreage	3.0	0.01	6.3	**0.01**	2.2	0.18	**7.0**	**0.005**
ECA	**3.1**	**0.1**	1.0	0.62	3.3	0.14	**5.6**	**0.01**
Vegetative barrier	2.2	0.19	1.2	0.49	0.4	0.82	**1.9**	**0.24**
Topographic barrier	0.9	0.55	1.8	0.38	**4.9**	**0.038**	0.5	0.93
Structural barrier	0.6	0.76	1.6	0.45	**4.7**	**0.043**	0.4	1.0
Presence of pet	3.1	0.1	3.1	0.12	1.0	0.61	0.4	0.96
Pet treatments	1.9	0.28	1.9	0.28	1.0	0.57	0.5	0.99
Outdoor domestic treatments	0.3	0.86	0.3	0.86	0.1	0.98	0.5	0.94
Indoor domestic treatments	**5.2**	**0.013**	5.2	**0.013**	2.5	0.19	1.0	0.65
Number of inhabitants	0.0	1	0.0	1	0.7	0.68	1.1	0.71

%var: percentage of variability of the exposure to agricultural pesticides explained by the variable; ECA: Effective Contributing Area for pesticide drifts. Results were obtained using redundancy analyses (RDA). P-value was estimate using permutation tests. Variables retained in the multivariate models using forward stepwise approach (threshold p=0.01) are presented in bold.

Table S4. Variability of the agricultural pesticide contamination explained by the cop acreage for different buffer size based on old dust samples

	Orchards (Zone 1)		Cereals (Zone 1)		Cereals (Zone 2)		Vineyards (Zone 3)	
	%var	p-value	%var	p-value	%var	p-value	%var	p-value
250m	8.1	0.005	6.0	0.01	1.7	0.36	9.0	0.005
500m	**12.7**	**0.005**	6.8	0.005	0.2	0.95	10.1	0.005
750m	9.3	0.01	4.7	0.01	0.3	0.97	7.1	0.005
1000m	6.0	0.005	**7.5**	**0.005**	**3.0**	**0.14**	**10.6**	**0.005**
1250m	5.2	0.005	5.1	0.017	0.4	0.93	10.3	0.005

%var: percentage of variability of the exposure to agricultural pesticides explained by the variable. Results were obtained using distance-based redundancy analyses. P-values were estimate using permutation tests. Analyses were made on qualitative data from recent dust samples. Buffer size retained for subsequent on old dust samples are presented in bold.

Table S5. Potential determinants of the agricultural pesticide exposure based on univariate analyzes of qualitative measures in old dust samples

	Orchards in Zone 1 (500m)		Cereals in Zone 2 (1000m)		Cereals in Zone 2 (500m)		Vineyards in Zone 3 (1000m)	
	%var	p-value	%var	p-value	%var	p-value	%var	p-value
Crop acreage	12.7	0.005	**7.5**	**0.005**	**3.0**	**0.12**	**10.7**	**0.005**
ECA	**12.5**	**0.005**	2.3	0.19	2.6	0.12	6.8	0.005
Vegetative barrier	0.6	0.94	**1.2**	**0.58**	**2.8**	**0.16**	1.1	0.63
Topographic barrier	1.4	0.47	2.5	0.14	2.7	0.12	3.5	0.045
Structural barrier	1.7	0.29	**2.5**	**0.11**	1.4	0.44	0.8	0.82
Presence of pet	4.6	0.017	5.0	0.005	1.1	0.49	1.0	0.71
Pet treatments	2.8	0.13	3.1	0.051	0.6	0.83	2.0	0.22
Outdoor domestic treatments	1.2	0.52	0.8	0.79	1.5	0.42	1.4	0.49
Indoor domestic treatments	**5.4**	**0.01**	**5.1**	**0.005**	**2.7**	**0.17**	**2.6**	**0.14**
Number of inhabitants	1.3	0.54	1.5	0.39	0.3	0.99	0.9	0.74

%var: percentage of variability of the exposure to agricultural pesticides explained by the variable; ECA: Effective Contributing Area for pesticide drifts. Results were obtained using distance-based redundancy analyses (db-RDA) on old dust samples. Variables retained in the multivariate models using forward stepwise approach (threshold p=0.01) are presented in bold.

Table S6. Variability explained by multivariates models in recent dust, after exclusion of potential clusters

	Models	Variable included in the models	% var
Orchards in Zone 1 (500m)	A	indoor domestic treatments + acreage	7.2
	B	acreage + ECA	9.0
	C	indoor domestic treatments + ECA	8.4
Cereals in Zone 1 (1000m)	A	indoor domestic treatments + acreage	12.4
	B	acreage + ECA	2.1
	C	indoor domestic treatments + ECA	11.9
Cereals in Zone 2 (500m)	A	topographic barriers + structural barriers	9.6
	B	-[a]	-[a]
	C	topographic barriers + structural barriers	10.2
Vineyards in Zone 3 (1000m)	A	acreage + vegetative barriers	15.1
	B	acreage + vegetative barriers	17.7
	C	acreage + vegetative barriers + ECA	14.1

%var: proportion of variability of the exposure to agricultural pesticides explained by the model.
[a]: analysis was not possible because only one agricultural compound was above 10% of detection.
Completes models are based on stepwise procedure. Models A are based on multivariate analyses of qualitative data (detection of pesticides either in dust trap of floor wipes). Models B are based on quantitative results for dust trap samples. Models C are based on quantitative results from floor wipes samples.

Table S7. Proportion of agricultural fields within 1000 meters for Zone 1, 2 and 3

Type of crops	Zone 1 (median + IQR[a])	Zone 2 (median + IQR[a])	Zone 3 (median + IQR[a])
Cereals	21.6% (12.9 – 30.2) [b]	21.5% (14.5 – 31.8) [b]	2.5% (0 – 9.8)
Peaches & Apricots	4.3% (2.0 - 10.5) [b]	0	0
Vineyards	0.1% (0 - 1.4)	0	29.7% (6.3 – 48.3) [b]
Cereals, organic	0% (0 – 0.4)	0% (0 – 1.2)	0
Fallow	0.2% (0 – 0.7)	0.9% (0.1 – 1.8)	0% (0 – 0.2)
Leguminous	0	2.7% (0 – 3.2)	0
Meadow	2.9% (0.7 – 4.4)	5% (0 – 9.1)	7.4% (3.1 – 16.5)
Meadow, organic	0	0% (0 – 0.3)	0% (0 – 0.3)
Other orchards	0.6% (0 – 1.9)	0	0% (0 – 0.1)
Sunflower & rape	4.5% (0.09 – 7.4)	3.6% (1.2 – 4.9)	0% (0 – 1.7)
Vegetables	0.2 (0 – 2.8)	0	0
Vineyard, organic	0	0	0% (0 – 0.8)
Various	0.5% (0 - 2.2)	0% (0 – 0.2)	0.2% (0 – 0.6)
Undefined	0.3% (0 – 0.5)	0.5% (0 – 1.3)	0

[a]: Interquartile range; [b]: Targeted crops considered in analyzes.

Chapter IV:
Development of the TESTIS project

Feasibility and development of a case-control study designed to explore the association between prenatal exposure to pesticide and testicular germ cell tumor during adulthood

IV.1 Synthèse en français / summary in English

SYNTHESE – FRANÇAIS

Objectif du chapitre : Avant de mettre en place une vaste et coûteuse étude cas-témoins, nous avions besoin de vérifier notre aptitude à recruter les sujets (cas et témoins) et leurs mères (pour obtenir des informations sur les périodes prénatales), ainsi qu'à collecter les informations nécessaire pour évaluer les expositions d'intérêt. Le taux de participation est un point clé de notre étude dans la mesure où le niveau socio-économique (SES) a été suggéré comme facteur de risque de TGCT. Dans la mesure où il n'existe pas de registre exhaustif de la population jeune en France, il est difficile d'approcher cette population pour des études épidémiologiques, surtout pour des sujets afférents à la reproduction ou la sexualité – tels que les TGCT. Nous avions donc besoin de tester le recrutement de ces sujets dans cette tranche d'âge. D'autre part, la qualité des résultats obtenus via le GIS dépendent de la précision du géocodage, mais à notre connaissance, aucune étude épidémiologique française utilisant un GIS n'est remontée jusque dans les années 70. Une étude pilote était également nécessaire pour poser les bases du protocole de l'étude cas-témoins finale.

Etude pilote : L'étude pilote cas-témoins intitulée TESTEPERA (*Tumeur germinale du testicule : étude des expositions professionnelles et environnementales en région Rhône-Alpes*) a été conduite entre 2011 et 2012. Sur les 181 cas de TGCT traités au CLB en 2008 et 2010, seulement 70 (39%) ont rempli les critères d'inclusion : 71 (39%) ont été exclus car ils n'avaient pas entre 18 et 44 ans et 40 (22%) ont été exclus car ils étaient nés en dehors de la région Rhône-Alpes. Au total, 150 hommes ont été contactés : 58 cas traités au CLB et 92 témoins recrutés dans une maternité régionale de l'agglomération lyonnaise (conjoints de femmes suivies à la maternité). Nous avons testé différentes approches pour le recrutement des cas et des témoins. La participation variait de 33% pour les cas diagnostiqués en 2008 à 68% pour les cas diagnostiqués en 2010 (période de recrutement Septembre 2011 – Avril 2012). La participation des témoins variait également selon l'approche (13% pour ceux recrutés lors d'un entretien en face-à-face, 0% en cas de recrutement par téléphone uniquement, 50% en cas de recrutement en face-à-face combiné à une relance téléphonique). Sur 50 cas et témoins inclus, 38 ont autorisé que l'on recontacte leur mère (76%). Au total, 24 mères ont accepté de participer (67% des mères

contactées, 48% du total). Les participants ont tous été contactés par un enquêteur en aveugle de leur statut de cas ou témoin pour remplir un questionnaire standardisé administré par téléphone. Les données collectées ont permis l'identification des métiers des parents et des sujets, ainsi que le géocodage des adresses des sujets de manière précise (82% des adresses était considérées comme précises à moins de 200m). La précision du géocodage dépendait du niveau d'urbanisation ($p<0.001$) mais pas de l'ancienneté de l'adresse ($p=0.52$).

Ces résultats soulignent la nécessité de procéder à un recrutement prospectif des cas pour limiter les délais entre diagnostic et recrutement, ce qui semble être un facteur déterminant du taux de participation, que ce soit pour les cas ou leurs mères. La possibilité de recourir à un recrutement via des registres préexistants semble donc être à éviter, dans la mesure où cette approche implique un délai d'au moins un à deux ans. La moins bonne précision du géocodage semble être liée aux adresses rurales, où les rues n'ont pas de numéros. Ce dernier point pose problème, dans la mesure où il s'agit des secteurs les plus proches des cultures agricoles. Le questionnaire devra donc être aménagé pour permettre l'inclusion des données complémentaires visant à corriger manuellement les coordonnées géographiques du sujet. Enfin, si l'on considère la mobilité des sujets entre les régions, la rareté de la pathologie, et le besoin d'un recrutement prospectif, nos résultats nous amène à préférer une étude nationale à une étude régionale sur plusieurs années.

Choix du groupe témoin : Le choix du groupe témoins pour l'étude cas-témoins s'est avéré être une question délicate et a nécessité des recherche préalable. Nous avons étudié différentes approches, incluant la constitution d'un groupe représentatif de la population générale via l'annuaire ou les listes électorales, mais les listings étaient incomplets et les enquêtes téléphoniques ne donnent pas de bon résultat chez les hommes jeunes en France, comme nous l'avons vu dans l'étude pilote. L'utilisation de registre de l'Assurance Maladie a également été écartée dans la mesure où seule l'adresse postale est disponible. Recruter les sujets à l'hôpital permet d'améliorer le taux de participation, dans la mesure où le personnel soignant à une relation privilégiée avec les sujets. De plus, cela facilite la prise en charge des prélèvements biologiques (le plasma doit être centrifugé et congelé dans l'heure). Au vu des difficultés à recruter des hommes jeunes et du risque d'abandon lié aux prélèvements biologiques et à la taille

du questionnaire, nous avons décidé de proposer une compensation financière pour soutenir le taux de participation.

Protocole de l'étude cas-témoins finale (projet TESTIS) : Elaboré à partir des études TESTEPERA et SIGEXPO, il s'agira d'une étude nationale cas-témoins basée sur 500 cas (validés histologiquement) et 1000 témoins fertiles ou féconds. Les recrutements seront réalisés par l'intermédiaire du réseau des Centres d' Etude et de Conservation des Œufs et de Sperme humain (CECOS) pour les cas et des maternités régionales adjacentes pour les témoins. Les sujets et leurs mères seront interviewés par téléphone par des enquêteurs professionnels formés sur l'étude. Le recrutement et la saisie des données se feront via une plateforme sécurisée en ligne. Nous allons croiser différentes approches dans le but d'évaluer les expositions (professionnelles, environnementales et domestiques) aux pesticides pendant les périodes critiques du développement et sur les périodes de l'adolescence ou de la vie adulte (pour étudier l'hypothèse des expositions combinées). Un GIS sera utilisé pour évaluer les expositions environnementales, en tenant compte de l'histoire résidentielle des sujets, de l'occupation des sols, des vents dominants et de la présence de barrières végétales. Les expositions professionnelles seront déterminées par un hygiéniste industriel à partir des emplois et des tâches effectués par les sujets et par leurs parents. L'exposition domestique sera déterminée à partir des données auto-rapportées par les sujets et en ayant recours à un expert. Celui-ci pourra se baser sur la matrice américaine développée par le US National Cancer Institute (NCI). Dans une deuxième étape, l'évaluation des expositions professionnelles sera étendue à d'autre source de perturbateurs endocriniens, incluant les solvants, les métaux, les fumées de soudages, certains plastiques et la production ou l'utilisation de certains médicaments. Un prélèvement sanguin sera également proposé à chaque volontaire pour évaluer les polymorphismes génétiques connus pour être associés au risque de TGCT, ainsi que de potentielles interactions gènes-environnements. Notre étude a été construite de manière à optimiser la possibilité d'études poolées ultérieures, et la banque de données biologiques permettra la réalisation de futures études génétiques ou toxicologiques ancillaires.

SUMMARY – ENGLISH

Aim of the chapter: Before launching a large and expensive case-control study of TGCT, we needed to verify our ability to recruit subjects (both cases and controls) and their mothers (to get information on the prenatal period) and to collect the data needed to perform the GIS-based exposure assessments. The participation rate is an important feature in such a study, since socio-economic status (SES) has been suggested as a potential risk factor of TGCT. However, since no exhaustive population registry exists in France, young men from the general population are difficult to approach for epidemiological studies, especially for sensitive topics like TGCT. In addition, the quality of GIS-based exposure assessments depends to the precision of the geocoding, but, to our knowledge, a retrospective geocoding of subjects back to the 1970's has never been done for epidemiologic studies in France. A pilot study was also needed to optimize the protocol of the future case-control study.

Pilot study: The TESTEPERA case-control pilot study (French acronym for *"Testicular germ cell tumors: studying environmental and occupational exposure in the Rhône-Alpes region"*) was conducted between 2011 and 2012. Among the 181 TGCT cases treated at the CLB (referral center of the Rhône-Alpes region for TGCT) in 2008 and 2010, 70 (39%) fulfilled the inclusion criteria. Seventy-one (39%) were excluded because they were younger than 18 or older than 44 years, and 40 (22%) were excluded because they were born outside the Rhône-Alpes region. Overall, 150 male subjects were contacted in the Rhône-Alpes region: 58 cases from the CLB and 92 controls from a regional maternity in Lyon (male partners of women treated at the maternity). Cases and controls were recruited using different approaches to test the best design for recruitment. Participation rates varied from 33% for cases diagnosed in 2008 to 68% for cases diagnosed in 2010 (recruitment periods: September 2011 – April 2012). Participation rate of control subjects varied depending on modalities of contact (13% for face-to-face recruitment; 0% when contacted by phone only; 50% for face-to-face recruitment combined with a phone reminder). Out of the 50 cases and controls that agreed to participate, 38 agreed for contacting their mother. Among them, 24 mothers agreed to participate (67% of participant, 48% of the total). All participants were contacted by an interviewer, who was blinded to their case/control status, to complete a standardized phone questionnaire. Data collection allowed precise job identification and geocoding of subjects' addresses (imprecision were lower than 200m for 82%

of these). Precision of geocoding was dependent on the level of urbanization ($p<0.001$) but not on the time period ($p=0.52$).

These results highlight the need for a prospective recruitment of cases, limiting the delay between diagnosis and recruitment, which seems to be an important factor in determining the likelihood of participation, for both cases and their mothers. It excludes the possibility of a registry-based recruitment as this often implies at least one or two years of delay since diagnosis. The lower precision of geocoding is likely to be related to rural addresses, where hamlets don't use street names or house numbers. The GIS precision in these areas is of the highest importance since these areas should be the most proximate to agricultural crops. The questionnaire will be adapted to include additional information to allow precise location in these specific situations. Considering the mobility of subjects between the French regions, the rarity of the diseases and the need for prospective recruitment, our pilot study suggests that a national case-control design is the necessary and preferable design to adequately address this research question.

Choice of the control group: Given the age and gender of the study population, the choice of the control group for the case-control study was a sensitive topic and required prior investigations. We explored different approaches which included the constitution of a representative group of subjects using the phone book or the electoral listings, but these registers were incomplete and phone-based recruitment does not provide representative results in the young, French population, as seen in the pilot study. The use of the register of the national French health insurance has been discarded since only the mailing address is available. Recruiting subjects within hospitals would allow better participation rates, since subjects are more confident in the medical staff. Moreover, it allows easier handling of biological samples (plasma samples have to be centrifuged and frozen within one hour). Considering the difficulties to recruit young men and the risk of non-participation in the biological sample component and the size of the questionnaire, we decided to offer financial compensation to improve the participation rate.

Protocol of the final case-control study: The study design, developed through the TESTEPERA and the SIGEXPO study, is a nation-wide case-control study of 500 TGCT cases (ascertained through histology) and 1000 fertile/fecund age-matched male controls. The recruitment will be

made through the CECOS network (*Centres d'Etude et de Conservation des Œufs et de Sperme humain*) for cases and the associated regional maternity for controls: 21 out of the 23 French CECOS agreed to participate to the study. Trained professional interviewers will interview the subjects and their mothers by phone using standardized questionnaire. Recruitment and data collection will be made using an online secured platform. We will combine different approaches in order to assess pesticide exposures (domestic, occupational and environmental) during critical time periods of development and during adolescence / early adulthood (to assess combined early and later-life exposures). Our GIS-based approach to assess environmental pesticide exposure is based on a life-time residential history, land use information, prevailing winds and presence of vegetative barriers. Occupational pesticide exposures will be assessed by an industrial hygienist based on subjects' and parents' occupations and tasks. Domestic exposure will be based on self-reports (type of use) and expert-based assessments, based on a US matrix developed by the National Cancer Institute (NCI). Occupational exposure assessment will be extended to other groups of endocrine disruptors: solvents, metals, welding fumes, plasticizer and some medicines. A blood sample will be collected from each participant to assess genetic polymorphisms known to be associated with TGCT risk, as well as to explore potential gene-environment interactions. Our design has been optimized to allow future pooled studies, and the biological database will allow studying further genetic or toxicological hypothesis.

IV.2 (article #5)

Tumeurs germinales du testicule et expositions précoces aux pesticides : étude pilote TESTEPERA

Rémi Béranger[1, 2, 3], Jeffrey Blain[1], Cédric Baudinet[1], Elodie Faure[1], Aude Fléchon[4], Helen Boyle[4], Virginie Chasles[5], Barbara Charbotel[6], Joachim Schuz[2], Béatrice Fervers[1, 3]

(1) Unité Cancer et Environnement, Centre Léon Bérard, 28 rue Laennec, 69373 Lyon Cedex

(2) Section Environnement et Rayonnements, Centre International de Recherche sur le Cancer, 150 cours Albert Thomas, 69372 Lyon Cedex

(3) Université Claude Bernard – Lyon1, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex

(4) Département d'oncologie Médicale, Centre Léon Bérard, 28 rue Laennec, 69373 Lyon Cedex

(5) EA 4129 'Santé, Individu, Société', Université Jean Moulin - Lyon 3, 7 rue Chevreul, 69007 Lyon, France.

(6) UMRESTTE UMR T 9405, Université Claude Bernard - Lyon 1, 43 Boulevard du 11 Novembre 1918, 69622 Villeurbanne Cedex

Article published in *le Bulletin du Cancer*
(2014 Mar;101(3):225-35)

Article original
Original article

Bulletin du Cancer
Société Française du Cancer

Volume 101 • N° 3 • mars 2014

Tumeurs germinales du testicule et expositions précoces aux pesticides : étude pilote TESTEPERA

Testicular germ cell tumours and early exposures to pesticides: The TESTEPERA pilot study

Rémi Béranger[1,2,3], Jeffrey Blain[1], Cédric Baudinet[1], Élodie Faure[1], Aude Fléchon[4], Helen Boyle[4], Virginie Chasles[5], Barbara Charbotel[6], Joachim Schüz[2], Béatrice Fervers[1,3]

[1] Centre Léon-Bérard, Unité Cancer et Environnement, 28, rue Laennec, 69373 Lyon cedex, France

[2] Centre international de recherche sur le cancer, Section Environnement et Rayonnements, 150, cours Albert-Thomas, 69372 Lyon cedex, France

[3] Université Claude-Bernard Lyon 1, 43, boulevard du 11-Novembre-1918, 69622 Villeurbanne cedex, France <r.beranger26@gmail.com>

[4] Centre Léon-Bérard, Département d'oncologie médicale, 28, rue Laennec, 69373 Lyon cedex, France

[5] Université Jean-Moulin Lyon 3, EA 4129 « Santé, Individu, Société », 7, rue Chevreul, 69007 Lyon, France

[6] Université Claude-Bernard Lyon 1, UMRESTTE UMR T 9405, 43, boulevard du 11-Novembre-1918, 69622 Villeurbanne cedex, France

Article reçu le 18 novembre 2013,
accepté le 30 décembre 2013
Tirés à part : R. Béranger

Pour citer cet article : Béranger R, Blain J, Baudinet C, Faure É, Fléchon A, Boyle H, Chasles V, Charbotel B, Schüz J, Fervers B. Tumeurs germinales du testicule et expositions précoces aux pesticides : étude pilote TESTEPERA. *Bull Cancer* 2014 ; 101 : 225-35.
doi : 10.1684/bdc.2014.1901.

Résumé. **Position du problème.** Les tumeurs germinales du testicule (TGCT) sont le principal cancer de l'homme jeune. Des facteurs environnementaux survenant au cours des périodes prénatales sont suspectés, mais très peu d'études ont été conduites.
Méthodes. TESTEPERA est une étude pilote cas-témoins visant à évaluer différentes approches pour le recrutement des sujets en France ainsi que notre capacité à recueillir les données nécessaires pour évaluer leurs expositions prénatales.
Résultats. Entre 2011 et 2012, 150 hommes ont été contactés en Rhône-Alpes (58 cas dans un centre de lutte contre le cancer et 92 témoins dans une maternité régionale). Les taux d'acceptation des cas variaient de 33 % pour ceux diagnostiqués en 2008 à 68 % pour ceux diagnostiqués en 2010. Le taux d'acceptation des témoins était de 13 % pour ceux rencontrés physiquement, 0 % pour ceux contactés par téléphone uniquement, et 50 % pour ceux rencontrés physiquement et relancés par téléphone. Les données recueillies ont permis l'identification des métiers exercés. Quatre-vingt-deux pour cent des adresses étaient géolocalisées précisément. La précision dépendait du niveau d'urbanisation ($p < 0{,}001$), mais pas de l'ancienneté ($p = 0{,}52$).

Abstract. **Background.** Testicular germ cell tumors (TGCT) represent the most frequent cancer in men aged between 15 and 45 years. Current hypotheses are focusing on environmental exposures occurring during prenatal periods. However, very few studies have explored intra-uterine environmental exposure related to TGCT. **Methods.** TESTEPERA is a pilot case-control study aiming to determine the effectiveness of different recruitment approaches in the French context and to verify our ability to collect relevant data on their prenatal periods. **Results.** Between 2011 and 2012, 150 male subjects were contacted in the Rhône-Alpes region (58 cases from a cancer center and 92 controls from a regional maternity). Participation rate varied from 33% for cases diagnosed in 2008 vs 68% for cases diagnosed in 2010. Participation rate of controls varied depending on modalities of contact (13% for face-to-face recruitment; 0% for contact by phone only; 50% for face-to-face contact with phone reminder). Data collection allowed precise job identification and geolocation of subjects' addresses. Precision of geolocation was dependent upon the level of urbanization ($p < 0.001$) but not on the time period ($p = 0.52$). **Conclusion.** Our

doi : 10.1684/bdc.2014.1901

Conclusion. Nos résultats confirment la faisabilité d'une étude cas-témoins étudiant la relation entre les TGCT et l'exposition environnementale précoce ou tardive aux pesticides. ▲

Mots clés : tumeurs germinales du testicule, étude pilote, expositions environnementales, système d'information géographique, pesticides

results support the feasibility of a case-control study focusing on the relation between TGCT and environmental pesticide exposures during early and later life. ▲

Key words: testicular germ cell cancer, feasibility study, environmental exposures, geographic information systems, pesticides

Introduction

Les tumeurs germinales du testicule (TGCT, fréquemment appelées cancer du testicule) sont les tumeurs les plus fréquentes chez l'homme de 15 à 45 ans. En France, leur taux d'incidence a augmenté de 2,5 % par an entre 1980 (3,4/100 000 personnes-années) et 2005 (6,4/100 000) [1], et les projections de l'Institut de veille sanitaire pour 2008 sont de 7 pour 100 000, soit un doublement de l'incidence en 30 ans [2]. L'augmentation rapide de l'incidence au niveau national et international, l'existence de variations géographiques de l'incidence [2, 3] et l'évolution de l'incidence chez les migrants [4, 5] sont en faveur d'un rôle de facteurs environnementaux dans l'étiologie des TGCT.

Plusieurs éléments étayent l'hypothèse d'une origine prénatale de ces tumeurs : le jeune âge des malades, le fait que les principaux types tumoraux de TGCT dérivent du *carcinoma in situ* (aussi appelé néoplasie germinale intratubulaire) dont la présence chez les jeunes enfants est connue [6], et l'association entre TGCT, cryptorchidie et hypospadias (deux malformations congénitales) [7]. L'hypothèse d'un « syndrome de dysgénésie testiculaire », regroupant certaines formes d'infertilités masculines, les cryptorchidies, les hypospadias et les TGCT a été avancée [7], mais reste controversée [8, 9]. Une des causes pourrait être liée à l'action de perturbateurs endocriniens œstrogéniques ou anti-androgéniques [10]. L'hypothèse d'un développement de la maladie en deux temps, associant altération intra-utérine et expositions au moment de la puberté ou chez le jeune adulte, a également été avancée [11, 12].

Si de nombreux facteurs professionnels et environnementaux ont été étudiés, notre revue de la littérature montre que seulement neuf études [13-21] ont exploré l'impact des expositions précoces de la vie, avec des résultats discordants [22]. Quatre de ces études étaient basées sur des estimations indirectes ou approximatives des expositions [16, 18, 19, 21], au moins trois semblaient souffrir d'un manque de puissance [13, 14, 21], et seulement deux prenaient en compte les expositions liées à l'environnement (habitat en milieu rural ou urbain) ou aux habitudes domestiques (exposition à des perturbateurs endocriniens ou des pesticides) [14, 20]. L'augmentation de l'incidence des TGCT, le manque d'informations disponibles et fiables, le jeune âge des sujets et le caractère potentiellement évitable des expositions environnementales justifient la réalisation de nouvelles études, y compris en France.

Compte tenu de la faible incidence des TGCT et de leur temps de latence, aucune des cohortes françaises existantes ne permet d'étudier cette pathologie et l'étude cas-témoins constitue l'approche la plus appropriée. Cependant, le recrutement d'une population masculine de 18 à 45 ans s'avère souvent délicat, ces sujets étant potentiellement plus difficiles à approcher et à motiver. De plus, les hommes de cette tranche d'âge sont souvent mobiles, ce qui rend plus complexe l'étude des expositions survenues dans une zone géographique définie (département, région), plusieurs décennies avant l'exposition. Enfin, les TGCT touchant à des domaines sensibles tels que la fertilité et la sexualité des sujets, les approches classiques concernant le recrutement des sujets peuvent ne pas être toutes adaptées.

Une autre difficulté concerne l'évaluation des expositions pendant la période périnatale, en particulier les expositions environnementales. Les systèmes d'informations géographiques (SIG) sont de plus en plus utilisés pour estimer le niveau d'exposition aux pesticides agricoles, en confrontant les adresses des sujets aux sources d'expositions spatialisées [23-25]. Mais cette approche nécessite de pouvoir géolocaliser précisément l'adresse des sujets et de disposer de bases de données spatialisées concernant les expositions d'intérêt.

Dans ce contexte, l'étude pilote TESTEPERA (tumeurs germinales du TESTicule : étude des Expositions Professionnelles et Environnementales en Rhône-Alpes) avait pour objectif de déterminer la meilleure approche de recrutement des sujets, en France, pour une étude cas-témoins visant à étudier les expositions environnementales et professionnelles associées aux TGCT, y compris pendant la période prénatale. TESTEPERA visait également à évaluer la qualité du recueil de données auprès des sujets et de leurs parents. La précision du géocodage rétrospectif des adresses devait nous permettre d'apprécier la pertinence de l'utilisation d'un SIG pour reconstituer les expositions environnementales des sujets aux pesticides sur de longues périodes.

Population et méthodes

Il s'agissait d'une étude pilote de type cas-témoins monocentrique, coordonnée par l'unité cancer et environnement du Centre Léon-Bérard (CLB) et par la section environnement et rayonnements du Centre international de recherche sur le cancer (CIRC).

Population

Les critères d'inclusion des cas (patients présentant une TGCT confirmée histologiquement, hors séminomes spermatocytaires, tumeurs vitellines et tératomes immatures) et des témoins (sujets n'ayant jamais eu de TGCT) comprenaient les hommes âgés de 18 à 44 ans au diagnostic (cas) ou au recrutement (témoins), nés en région Rhône-Alpes. Les hommes ne parlant pas le français, présentant une pathologie psychiatrique ou un trouble mental sévère étaient exclus de l'étude.

Entre septembre 2011 et avril 2012, deux séries de cas ont été recrutées au CLB, Centre de lutte contre le cancer (CLCC) de la région Rhône-Alpes :

– série A : patients ayant une TGCT diagnostiquée en 2008 ;

– série B : patients ayant une TGCT diagnostiquée en 2010.

Les patients étaient contactés via un courrier co-signé par le médecin référent au CLB et le responsable de l'étude. Le courrier contenait une lettre d'information présentant l'étude, un formulaire de consentement avec une enveloppe T, et un document d'aide à la préparation de l'interview, concernant notamment les questions relatives à l'histoire résidentielle et professionnelle. Une relance téléphonique était réalisée en cas de non-réponse au bout de 3 semaines (jusqu'à 3 tentatives à une semaine d'intervalle, à des heures différentes de la journée).

Entre août et octobre 2012, trois séries de témoins ont été recrutées à la maternité de l'Hôpital de la Croix-Rousse, hôpitaux civils de Lyon, parmi les conjoints de femmes hospitalisées en suite de couches :

– série 1 : sujets approchés (face-à-face) par l'enquêteur qui leur présentait l'étude, mais sans relance téléphonique ;

– série 2 : sujets approchés par téléphone après remise des documents relatifs à l'étude à sa compagne, avec relance téléphonique (même procédé que pour les cas) ;

– série 3 : sujets approchés (face-à-face) par l'enquêteur, avec relance téléphonique.

Les témoins ont reçu les mêmes documents que les cas et devaient retourner le formulaire de consentement par voie postale à l'aide de l'enveloppe T.

Lorsque les cas et les témoins donnaient leur accord au niveau du formulaire de consentement, leur mère (ou le plus proche parent vivant) était contactée par courrier afin de leur proposer de participer à l'étude. Le courrier contenait le même type de documents qu'ont reçu les fils.

Recueil de données

Les informations relatives aux facteurs étudiés chez les sujets (cas et témoins) et leurs mères étaient recueillies à l'aide d'un questionnaire standardisé administré par téléphone, par un enquêteur formé n'ayant pas connaissance du statut cas ou témoins des participants de l'étude. Le recueil de données comprenait les items suivants : les données sociodémographiques, les antécédents familiaux et médicaux, les habitudes de vie, l'historique résidentiel et professionnel détaillé, l'exposition à certaines nuisances professionnelles identifiées dans la littérature (pesticides, fumées de soudage, solvants, matières plastiques) et les utilisations domestiques de pesticides. Le questionnaire « mère » comprenait des items relatifs aux pères (historique et nuisances professionnelles, antécédents médicaux). L'historique professionnel et résidentiel reposait sur des items simples et objectifs (intitulé du métier, tâches effectuées, différentes adresses de résidence), le but étant de permettre leur interprétation par des experts ou via l'utilisation de SIG. Les items relatifs aux expositions domestiques étaient dérivés du questionnaire utilisé dans l'étude CEREPHY, avec l'autorisation du professeur Lebailly [26]. Construit sur la même base, un questionnaire spécifique était proposé aux mères. Les interviews étaient conduites par téléphone, par des enquêteurs en aveugle du statut cas ou témoins des sujets.

Géocodage des adresses

L'ensemble des adresses de résidence a été répertorié, pour les sujets (logements occupés avant 18 ans) et leurs parents (au moment de la grossesse et l'année précédant celle-ci). Les adresses des établissements scolaires fréquentés pendant l'enfance par les sujets ont également été recensées. Le géocodage a consisté à affecter des coordonnées géographiques à l'adresse postale (format Lambert 93). La précision des adresses a été testée à l'aide d'un service web de géocodage permettant de classer les adresses selon leur niveau d'exactitude (www.batchgeocodeur.mapjmz.com/). Le niveau de précision varie de 0 à 9 : 0 en cas de localisation impossible ; 1 pour les adresses précises au niveau du pays ; 2 à la région ; 3 à la région secondaire (comté, municipalité) ; 4 à la ville, au village ou au hameau ; 5 au code postal de la commune ou de l'arrondissement ; 6 à la rue ; 7 à l'intersection de rue ; 8 à l'adresse exacte ; 9 sur un point d'intérêt (bâtiment, collège, centre commercial…). Les adresses de qualité inférieure ou égale à 6 ont toutes été réexaminées.

Bases de données pour l'évaluation des expositions environnementales

La construction du SIG pour évaluer les expositions environnementales nécessite de disposer d'éléments comme l'occupation du sol, les données météorologiques, le type de sol ou encore les données topographiques. Différentes bases de données régionales et nationales ont donc été recherchées afin de vérifier l'existence des données nécessaires à la construction de notre modèle pour une future étude cas-témoins.

Analyses

Le critère de jugement principal concernait le taux de participation (lettre de consentement signée). Les critères de jugements secondaires portaient sur la précision des adresses de résidence des sujets et de leur mère, ainsi que sur l'exhaustivité du recueil des données. Les taux d'inclusion et les caractéristiques des sujets ont été comparés à l'aide du test du Chi^2. La précision du géocodage des adresses a été comparée en fonction de la période et du niveau d'urbanisation à l'aide du test de Wilcoxon, compte tenu de la distribution des données. Les calculs ont été réalisés à l'aide du logiciel R (version 3.0.0). Le seuil de significativité retenu était de 5 % ($p < 0,05$).

Données éthiques

L'étude pilote a reçu l'avis favorable du Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé (CCTIRS, avis du 13 juillet, n° 11.267 bis) et l'accord de la Commission nationale de l'informatique et des libertés (CNIL, avis du 7 mars 2012, n° 911338). Les sujets participants ont été informés de leurs droits et ont signé un formulaire de consentement éclairé avant de participer.

Résultats

Modalités de recrutement des sujets et taux de participation selon les approches choisies

Sur les 181 cas de TGCT (hors séminomes spermatocytaires, tumeurs vitellines et tératomes immatures) suivis au CLB en 2008 ($n = 79$) ou 2010 ($n = 102$), 71 (39 %) étaient âgés de moins de 18 ans ou de plus de 44 ans au diagnostic. Quarante des 110 sujets restants (36 %) étaient nés hors de la région Rhône-Alpes, et 70 remplissaient les critères d'inclusion (39 % de l'effectif initial).

Au total, sur les 150 sujets contactés (58 cas et 92 témoins), 28 cas (48 %) et 22 témoins (24 %) ont accepté de participer. Le taux de participation était de 33 % pour les cas diagnostiqués en 2008, et de 68 % pour ceux diagnostiqués en 2010. Pour les témoins, le taux variait également selon les approches : 13 % en cas de contact direct sans relance, 0 % en cas de contact indirect avec relance téléphonique, 50 % en cas de contact direct avec relance téléphonique. L'écart observé était statistiquement significatif entre les groupes témoins ($p < 0,001$), mais pas entre les groupes cas ($p = 0,19$). Les *figures 1 et 2* détaillent les effectifs en fonction des approches.

Sur 50 sujets (cas et témoins) inclus, 38 ont autorisé que l'on recontacte leur mère (76 %). Au total, 24 mères ont accepté de participer (67 % des mères contactées, 48 % de l'ensemble des mères des sujets inclus). Le taux de participation pour les mères de cas diagnostiqués en 2010 était de 88 % contre 50 % pour celles de cas diagnostiqués en 2008. Concernant les mères de témoins, le taux de participation le plus important était celui du groupe recruté par contact direct avec relance téléphonique (60 %).

Recueil de données

Nos analyses ont porté sur 45 questionnaires « fils » (28 cas et 17 témoins) et 23 questionnaires « mère » (5 questionnaires « fils » et un questionnaire « mère » n'ont pu être complétés car les sujets n'étaient pas disponibles aux horaires de travail de l'enquêteur). La population de l'étude est présentée dans le *tableau 1*.

Les données du questionnaire ont permis d'identifier de manière satisfaisante les métiers et les expositions professionnelles déclarées. Au total, 188 métiers ont été relevés pour l'ensemble des sujets et 77 pour leurs parents pour les périodes d'intérêt. Les expositions déclarées par les sujets (pesticides, solvants, fumées de soudage ou composés plastiques) semblaient cohérentes avec les métiers déclarés. Trente pour cent des cas (8 sur 28) ont travaillé comme agriculteurs ou ouvriers agricoles, 22 % (6) ont déclaré une exposition aux pesticides, et 22 % (6) une exposition aux fumées de soudage. Des expositions professionnelles ont également été déclarées par les témoins (1 sur 17 pour les pesticides et 2 sur 17 pour les fumées de soudage) et les mères (sur les 23, 3 ont déclaré des expositions aux pesticides et aux fumées de soudage dans le couple sur la période prénatale). Des expositions aux solvants ont été fréquemment rapportées, mais renvoyaient à des familles de composés à la fois variées et hétérogènes (alcool, produit ménager, détergent, trichloréthylène...). L'exposition déclarée aux matières plastiques était plus rare (2 cas).

Qualité du géocodage des adresses des sujets

Pour les 45 sujets interrogés (cas et témoins), nous avons répertorié au total 100 adresses résidentielles occupées entre la naissance et l'âge de 18 ans (médiane 2, interquartiles 1-3), et 32 adresses résidentielles parentales pour les 23 mères interrogées (de un an avant la

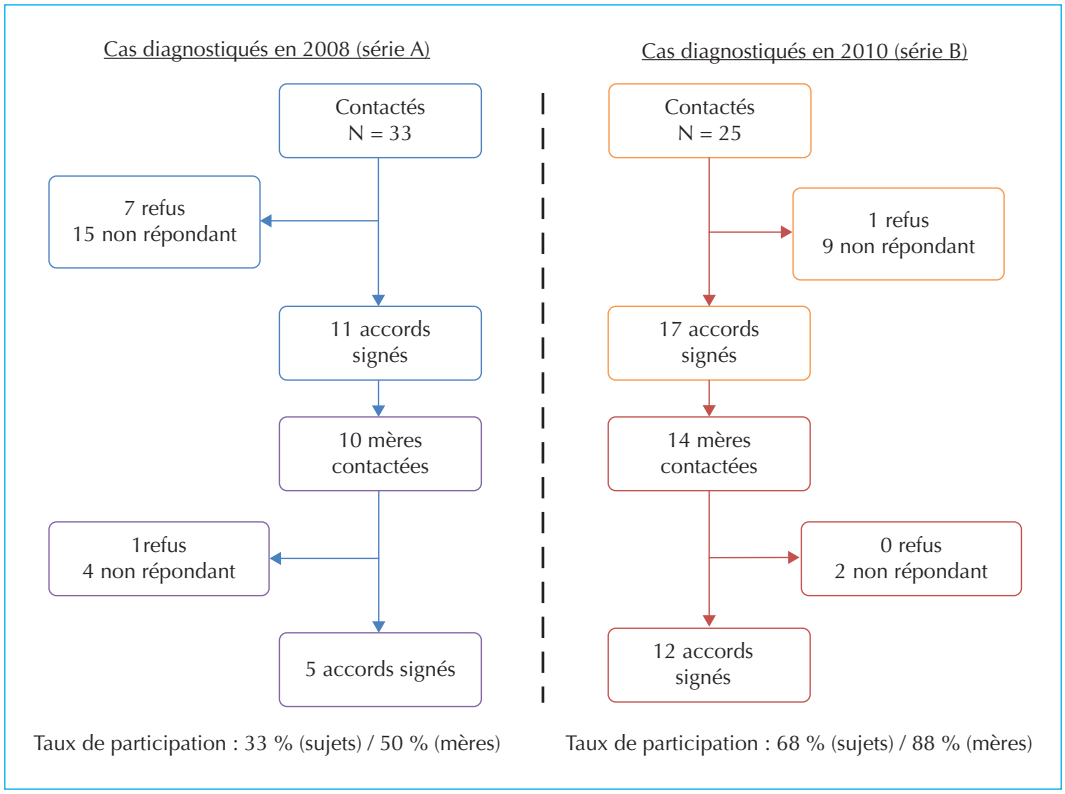


Figure 1. Diagramme d'inclusion des cas selon les différentes approches testées.

conception du sujet jusqu'à la fin de la grossesse). Une seule adresse est apparue divergente (numéro de rue) en comparant les données renseignées par les mères et les fils pour la résidence occupée au moment de la naissance. Nous avons privilégié les informations fournies par la mère.

Après examen des 77 adresses ayant un niveau de précision codé comme inférieur ou égal à 6, l'orthographe de 13 adresses a été corrigée et 2 adresses ont été complétées en croisant les questionnaires « mère » et « fils ». À l'issu de cette étape, 82 % des adresses étaient géolocalisées précisément (niveau de précision entre 6 et 9). Aucune adresse n'a été codée avec un score inférieur à 4 ou n'a pas pu être localisée. Pour 10 des 23 adresses codées 4 et 5, il s'agissait de lieux-dits ou de hameaux pour lesquels il n'existait pas de nom ou de numéro de rue.

Nous n'avons pas observé de différence statistique concernant le niveau de précision du géocodage des 132 adresses selon la période (1960-1979 *vs* 1980-2001 : score moyen 6,5 *vs* 6,7, $p = 0{,}52$). Pour les 109 adresses localisées en région Rhône-Alpes, le niveau de précision du géocodage était inférieur pour les communes de moins de 10 000 habitants par rapport aux communes de 10 000 habitants ou plus (score moyen 5,8 *vs* 7,1, $p < 0{,}001$). Les données relatives à la précision du géocodage sont présentées dans la *figure 3*.

Les établissements scolaires fréquentés par les sujets ont également été géolocalisés. Sur les 192 adresses répertoriées (43 écoles maternelles, 48 écoles primaires, 47 collèges et 54 lycées), 110 (57 %) étaient codées avec une précision de 6 ou plus, 81 (42 %) ont été attribuées au niveau 4 ou 5 (seuls le code postal et la commune étaient connus) et une adresse n'a pas pu être géolocalisée.

Bases de données pour l'évaluation des expositions environnementales

Le *tableau 2* décrit les principales bases de données identifiées pour la construction du SIG. Ces bases de données permettront d'étudier le mode d'occupation du sol et son évolution sur plusieurs décennies, ainsi que différents déterminants potentiels de l'exposition.

Discussion

À notre connaissance, l'étude pilote TESTEPERA est la première à tester et à comparer différentes approches pour le recrutement de cas et de témoins en France, pour l'étude des facteurs de risque environnementaux et professionnels des TGCT. Elle a mis en évidence de fortes disparités selon les différents modes de recrutement testés, en termes de taux de participation des sujets et de leur mère. Elle montre l'importance du délai entre le diagnostic de TGCT et le recrutement dans

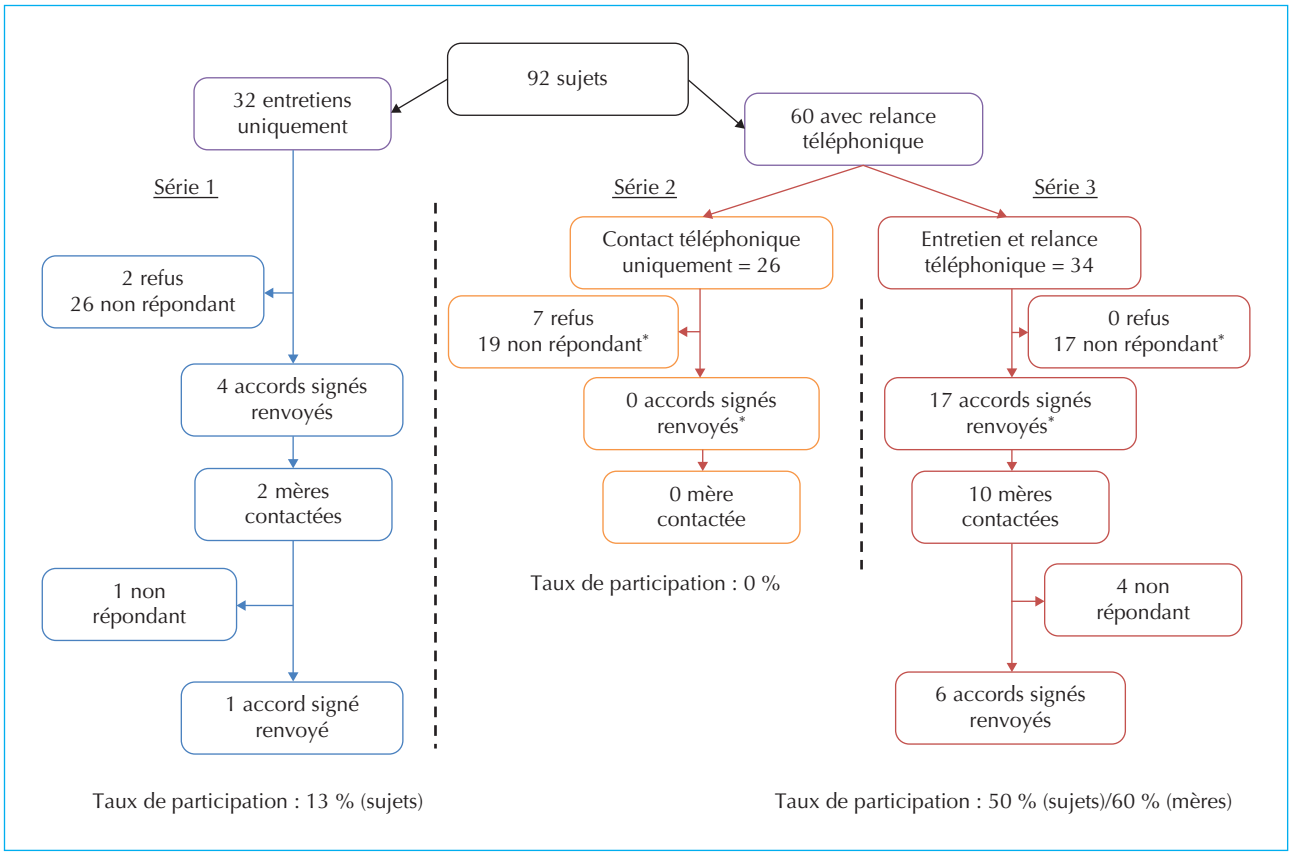


Figure 2. Diagramme d'inclusion des témoins selon les différentes approches testées. Dans la série 1, les sujets étaient recrutés directement (en face à face), sans relance téléphonique. Dans la série 2, les compagnes des sujets étaient approchées par l'enquêteur (distribution des documents relatif à l'étude), puis le sujet était contacté et relancé par téléphone. Dans la série 3, les sujets étaient recrutés directement et relancé par téléphone. *Parmi les 3 sujets ayant refusé de donner leurs noms et coordonnées à l'inclusion (série 2 ou 3), un sujet nous a transmis sa lettre de consentement signée, mais n'a pas pu être rattaché à un groupe. Il n'a donc pas été compté pour la réalisation de ce diagramme et fait partie des « non-répondants ».

l'étude pour les cas, mais aussi l'importance particulière d'une rencontre avec le recruteur et d'une relance téléphonique pour le recrutement des témoins dans cette population. Les taux de participation restent toutefois satisfaisants dans les situations les plus favorables. Cette étude confirme notre capacité à géolocaliser précisément les adresses des sujets pendant l'enfance à partir d'un questionnaire administré, condition nécessaire à l'utilisation d'un SIG.

Concernant le recrutement des cas et des témoins, les différents sous-groupes ont été choisis pour tester différentes approches possibles pour le recrutement. Pour les cas, une des solutions envisagées était de procéder à un recrutement rétrospectif à partir d'un ou plusieurs registres combinés : registre départemental du cancer, bases de données du programme de médicalisation des systèmes d'information (PMSI), CRISAP (centres de regroupement informatique et statistique en anatomie et cytologie pathologiques) ou registres hospitaliers. Le délai nécessaire au recueil et traitement des données dans ces bases de données implique un délai entre le diagnostic et l'inclusion et impose de contacter les cas par téléphone ou courrier. Nos résultats montrent que le taux de recrutement diminuait sensiblement avec l'augmentation du délai entre le diagnostic et le recrutement, même si l'écart observé n'est pas statistiquement significatif, probablement du fait du faible nombre de sujets. Un mode de recrutement prospectif nous paraît donc préférable. Le taux de participation des cas dans notre étude était comparable à ceux observés pour des études similaires aux États-Unis (68,5 et 72 %) [27, 28], en Italie (57 %) [20], au Royaume-Uni (55 %) [29], en Suède (78 %) [30] ou en Allemagne [31]. Une seule étude américaine obtenait des taux de participation supérieurs (80 %) [17]. La seule étude épidémiologique française publiée sur les TGCT était basée sur un recrutement prospectif des cas via les centres d'étude et de conservation d'œuf et de sperme humain (CECOS), au moment de leur prise en charge, et avait obtenu un taux de participation de 80 % (62 % après soustraction des perdus de vue et des sujets exclus) [32]. Dans la mesure où les cas de notre étude étaient recrutés uniquement par courrier, le taux de participation pourrait potentiellement être amélioré

Tableau 1. Présentation de la population d'étude.

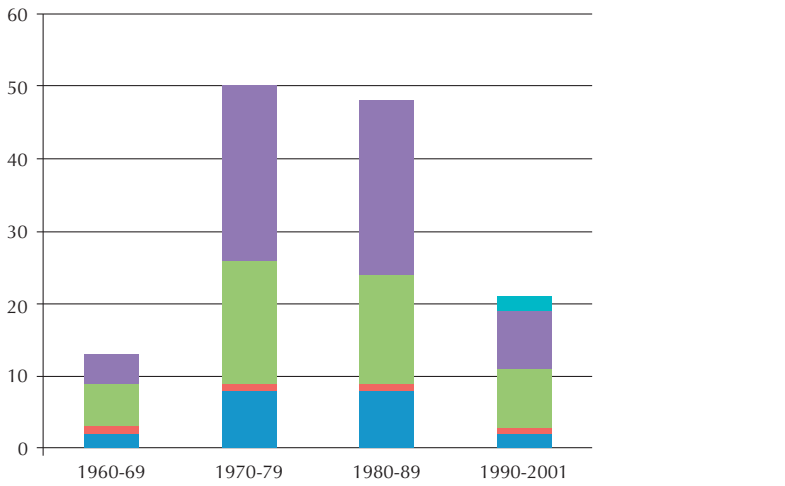
	Cas ($n = 28$)	Témoins ($n = 17$)	Valeur de p
Âge			0,25
18-24	1	0	
25-34	13	12	
35 et plus	14	5	
Niveau de diplôme			0,03[a]
Brevet/primaire	5	0	
Baccalauréat/secondaire	9	2	
BTS/IUT/DEUG/Licence	10	7	
2e ou 3e cycle	4	8	
Revenu mensuel du ménage			0,03[a]
Moins de 1000 euros	4	0	
1000-2000 euros	3	0	
2000-3000 euros	6	1	
Plus de 3000 euros	14	16	
Chômage			0,49
Oui	7	2	
Non	21	15	
RSA			0,99
Oui	3	1	
Non	25	16	

[a] Différence statistiquement significative ($p < 0,05$).

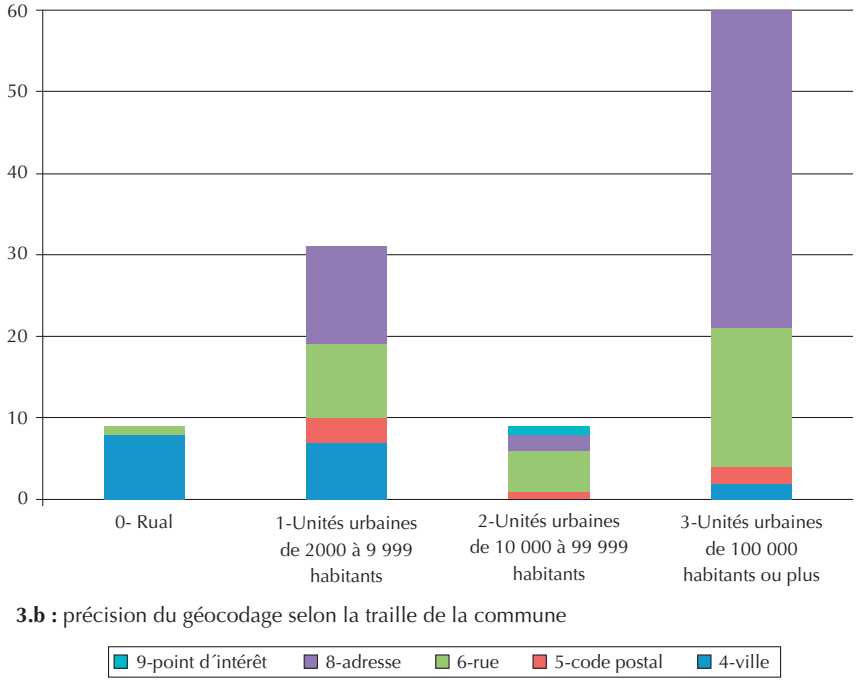
par un recrutement en face-à-face au moment de leur venue dans les centres de soins.

Selon l'hypothèse du syndrome de « dysgénésie testiculaire », les TGCT, les cryptorchidies, les hypospadias et certains troubles de la fertilité correspondent à une même altération prénatale, mais avec une expression clinique différente. Le choix de témoins féconds, recrutés à la maternité, permettrait de réduire le risque d'avoir des témoins atteints de formes mineures de ce syndrome. Nous avons choisi de ne pas exclure la cryptorchidie, fréquemment considérée comme facteur de confusion, afin de pouvoir mesurer son impact sur les modèles statistiques (avec ou sans ajustement) dans le volet final de l'étude. La population témoin devait également être représentative des cas sur le plan géographique, de manière à éviter la surreprésentation de sujets urbains ou ruraux dans une étude portant sur les expositions environnementales, d'où le choix d'une maternité de niveau III ayant un recrutement régional. Les différentes approches choisies pour le recrutement des témoins ont mis en avant l'intérêt d'un contact direct avec les sujets combinés à une relance téléphonique. Le taux de participation de notre étude pour la série 3 (recrutement face-à-face avec relance téléphonique) semble supérieur à la précédente étude française portant sur les TGCT (recrutement en face-à-face en maternité : 39 % de participation, 30 % après soustraction des perdus de vue et des sujets exclus) [32]. Une étude allemande sur le TGCT rapportait un taux de participation proche du nôtre pour le recrutement des témoins (57 %) [31]. En revanche, des taux supérieurs ont été observés pour trois études américaines (60,5-66 %) [17, 28, 33] et pour une étude suédoise (71 %) [30].

Les expositions prénatales présentant un intérêt majeur dans l'étude des TGCT, il nous a semblé pertinent d'inclure les mères des sujets. Ces dernières sont susceptibles d'apporter des informations complémentaires concernant les expositions prénatales ou datant de la jeune enfance du sujet. Dans une étude américaine, publiée en 1997, une approche similaire a été tentée dans le champ du cancer du testicule : sur les 495 cas et 974 témoins, respectivement 71 % et 61 % ont accepté que l'on contacte leur mère, 69 % et 54 % des mères ont finalement participé à l'étude [17]. Dans l'étude italienne publiée en 2006, 61 % des mères ont pu être interrogées (63 mères pour 103 sujets : 28 sujets ont refusé que l'on contacte leur mère, 12 des mères étaient décédées ou inconnues) [20]. Dans notre étude, le taux d'acception des sujets concernant une prise de contact avec leur mère est comparable aux données de la littérature. Si le taux de participation global des mères de notre étude est plus faible que dans la littérature, il est toutefois supérieur pour les voies de recrutement les plus performantes. Cependant, compte tenu du faible nombre de mères incluses, les chiffres doivent être considérés avec prudence. Des éléments relatifs aux parents pourraient également être incorporés dans le questionnaire des fils, afin d'avoir une base de renseignements minimums quant à leurs expositions (exemple : métier, adresse, pathologies particulières) en cas de refus de participation de la mère. La fiabilité de cette approche mériterait toutefois d'être testée au préalable.



3.a : précision du géocodage selon la période



3.b : précision du géocodage selon la traille de la commune

Figure 3. Précision du géocodage des adresses. 3.a : Les données concernant la précision du géocodage portent sur l'ensemble des 132 adresses répertoriées. 3.b : Les données concernant la précision du géocodage selon la taille de la commune en termes de nombre d'habitants portent sur les adresses localisées en région Rhône-Alpes uniquement (n = 109).

La mobilité des sujets entre le lieu de naissance (exposition) et le lieu de diagnostic ou de suivi pose deux problèmes distincts :
– une fuite des sujets nés sur le secteur d'étude, ce qui implique un biais de sélection ;
– la nécessité d'exclure les sujets nés sur des secteurs extérieurs en cas d'étude portant sur les expositions environnementales, ce qui réduit le nombre de sujets disponibles.

Les deux premières régions en termes d'occurrence des TGCT sont la région Île-de-France et la région Rhône-Alpes, avec une incidence annuelle respective d'environ 350 et 200 cas sur la période 2004-2008 [2]. Cependant, si l'on soustrait les sujets d'âge ou d'histologie inadéquats (39 % dans notre étude, 36 % des TGCT en France [2]), les sujets nés hors de la région (estimés à 22 % du nombre total de cas dans notre étude), ou encore les sujets suivis par des centres ou des

Tableau 2. Principales bases de données disponibles pour la construction du SIG.

Nom des données	Producteur de données	Échelle d'usage	Date de disponibilité	Disponibilité
Corine Land Cover	IFEN	1/100 000	1990/2000/2006	Nationale
Parcelles du RPG	DRAAF/DDAAF	1/5 000	Depuis 2006	Nationale
Recensement statistique agricole	DRAAF	Commune	1970-1979-1988-2000-2010	Nationale
Champs biologiques	DRAAF	1/5 000 au 1/25 000	Depuis 2006	Nationale
Vergers de types prunus	DRAAF	1/5 000	Depuis 2008	Nationale
Casier viticole automatisé	DGDDI-FranceAgriMer	1/5 000	2011-2012	Nationale
Type de sol	Chambre régionale de l'agriculture	1/100 000		Nationale
BD Topo	IGN	1/10 000	2012	Nationale
BD Ortho	IGN	1/10 000	2010	Nationale
BD Alti	IGN	25 m	2010	Nationale
BD Parcellaire	IGN	1/2 000	2010	Nationale
Point Adresse	IGN	1/10 000	2010	Nationale
BD Carto	IGN	1/50 000	1995	Nationale
Aire urbaine	Insee	Commune	2010	Nationale
Unité urbaines	Insee	Commune	2010	Nationale
Vents et pluie	Météo France	8 km	Journalier	Nationale
Rose des vents	Météo France	Station	Journalier	Nationale

médecins ne participant pas à l'étude, la population disponible serait considérablement réduite. La réalisation d'une étude régionale visant à recruter des effectifs importants (au moins 500 cas) sans avoir recours à un recrutement rétrospectif paraît alors difficile. Il semble préférable d'étendre la zone d'étude à la France entière. En plus de garantir une puissance suffisante, cette approche apporterait une solution à la mobilité suprarégionale des sujets, tout en permettant d'étudier les facteurs environnementaux potentiellement liés aux variations interrégionales [2,11]. L'utilisation d'un réseau préexistant (fédération des CECOS ou des CLCC) permettra de limiter les contraintes organisationnelles liées à la multiplication des centres.

L'évaluation rétrospective des expositions professionnelles est courante en épidémiologie, notamment dans les études sur le cancer où les temps de latence sont généralement longs. Dans ce contexte, la méthode de référence reste le recours à des experts se basant sur l'historique professionnel couplé à un descriptif des expositions et des conditions de travail [34]. Malgré le manque de détails parfois observé dans le descriptif des postes et des tâches effectuées, les données collectées ont permis d'identifier le métier et l'activité de l'entreprise. La précision et le niveau de détail des métiers et tâches peuvent être améliorés par la formation des enquêteurs.

Plusieurs études américaines ont géocodé des adresses rétrospectivement en se basant sur l'historique résidentiel [35-37] ou l'adresse à la naissance [38-40] : les taux de géocodage considérés comme précis (à l'adresse) allaient de 83 à 94 %. Dans l'article de Brody *et al.*, la précision des adresses semblait décroître avec l'ancienneté [35]. Les autres articles ne présentaient pas de données assez détaillées pour interpréter l'impact de la période sur la qualité du géocodage. En France, l'étude GEOCAP a permis de géocoder 2 779 adresses récentes (de 2002 à 2007), avec 80,9 % des adresses précises à moins de 100 mètres, 16,1 % précises de 300 à 500 mètres, et 3 % précises à la commune [41]. Si la précision de notre géocodage s'est avérée légèrement moindre que dans les études américaines, elle est très similaire à celle observée dans l'étude GEOCAP, malgré des adresses plus anciennes dans notre étude (1960-2001). Le degré d'ancienneté des adresses n'a pas semblé affecter la précision du géocodage. Le géocodage des adresses correspondant aux communes plus petites (moins de 10 000 habitants) a été moins précis, notamment en raison des adresses postales rurales ne comprenant pas de nom ni de numéro de rue. Toutefois, le fait de localiser une adresse au centroïde d'un hameau ou d'un lieu-dit ne semble pas induire une imprécision trop importante (estimée à 300 m dans l'étude GEOCAP) [41]. La précision du géocodage nous est apparue satisfaisante pour pouvoir étudier les expositions environnementales des sujets de manière fine (à la parcelle). Elle pourrait être affinée pour les adresses rurales en intégrant dans le questionnaire un item portant sur des éléments identifiables situés à proximité de la résidence, tels que les noms de routes qui se croisent ou un point d'intérêt notable. Le géocodage des établissements scolaires pourrait servir à compléter l'évaluation des expositions environnementales. Toutefois, la précision du géocodage était plus faible pour ce type d'adresses, une partie des sujets ne se souvenant que du nom et de la commune de l'établissement. Un travail de recherche manuel permettrait d'améliorer le niveau de précision dans un certain nombre de cas

(recherche dans les pages jaunes, appel à la mairie, rappel du sujet). Dans ce cas, le niveau de précision dépendrait de l'investissement mis en place.
Au cours des dernières décennies, le territoire agricole français a connu une artificialisation croissante, avec un rapprochement des populations aux zones d'application des pesticides. Les espaces périphériques des aires urbaines sont les plus concernés, aux alentours des villes, le long des réseaux routiers et des vallées [42]. Il est donc nécessaire de disposer de données sur l'occupation des sols sur l'ensemble de la période d'étude. Or, la base de données CORINE® Land Cover n'est disponible que jusqu'en 1990, et elle ne s'avère pas aussi précise que celles produites par la DRAAF ou les chambres d'agricultures à partir de 2005 (difficulté pour identifier les petites surfaces agricoles, échelle 1/100 000). Des méthodes permettant de reconstituer les données d'occupation du sol seront donc nécessaires, pour compléter (1990-2005) ou pour remplacer (avant 1990) les bases de données existantes. Des équipes américaines ont utilisé avec succès des images satellites infrarouges pour reconstituer les cultures présentes sur un territoire défini [43, 44]. Ces techniques de télédétection combinées à des photographies aériennes et les données du recensement statistique agricole ou des interviews d'experts, devraient permettre de pouvoir reconstituer les données d'occupation du sol de manière fiable. Les images satellites Landsat® sont disponibles dès 1972. Le SIG que nous avons développé pour caractériser les expositions environnementales aux pesticides a été construit à partir de mesures de pesticides réalisées dans des poussières domestiques de 239 foyers volontaires (projet SIGEXPO, données en cours de publication). Ce SIG se base sur les différentes bases de données identifiées dans le *tableau 2*.
Outre l'effectif modeste, mais usuel pour une étude pilote, certaines limites sont toutefois à prendre en compte dans notre étude. L'objectif de cette étude pilote n'était pas d'assurer une représentativité des sujets par rapport à la population générale, ni de permettre la comparabilité entre les cas et des témoins. Plusieurs points peuvent ainsi être associés à un biais de sélection : le recrutement des cas et des témoins était limité au niveau de deux centres, les témoins avaient un niveau socio-économique globalement plus élevé que les cas et la proportion de cas ayant travaillé en milieu agricole était supérieure à la population générale (10 % de l'emploi total en 1988 : http://agreste.agriculture.gouv.fr/IMG/pdf/AGRIFRA07c-2.pdf). L'acceptation des sujets concernant un éventuel prélèvement biologique n'a pas été testée, ce qui pourrait diminuer le taux de participation des témoins dans une étude ultérieure. Toutefois, un recrutement hospitalier direct devrait limiter cet effet en permettant la réalisation de ces prélèvements sur place, au moment du recrutement, par le personnel soignant. Une indemnisation peut également être envisagée pour augmenter le taux de participation.
La présente étude pilote nous a permis de choisir le design le plus approprié pour étudier les expositions précoces aux pesticides, mais aussi de possibles expositions combinées (expositions prénatales combinées à des expositions chez l'adolescent ou l'adulte), potentiellement associées à un excès de risque de TGCT. Pour le volet final de l'étude (projet TESTIS), nous avons choisi un recrutement prospectif national dans le réseau des 23 CECOS français pour les cas, et un recrutement des témoins dans les maternités régionales adjacentes (recrutement direct avec relance téléphonique). L'évaluation des expositions se basera sur l'utilisation d'un système d'informations géographiques (exposition environnementale), d'ingénieurs hygiénistes (exposition professionnelle) et d'un questionnaire administré par téléphone. Un prélèvement sanguin est également prévu pour étudier les interactions gènes-environnements. Le projet TESTIS a récemment reçu un financement de l'INCa et de l'Inserm.

Conclusion

À notre connaissance, cette étude est la première à tester et à comparer différentes approches pour le recrutement de cas et de témoins en France et dans ce contexte. Ce travail a permis d'orienter les choix méthodologiques pour la mise en place d'une étude cas-témoins visant à évaluer les facteurs environnementaux potentiellement associés avec le TGCT. Nos résultats montrent qu'un recrutement prospectif est à privilégier pour les cas et leurs mères, afin d'éviter des délais trop longs (plus d'un an) entre le diagnostic et le recrutement. Concernant le recrutement de témoins âgés de 18 à 44 ans, nos résultats tendent à montrer l'inefficacité des approches sans recrutement en face-à-face ou sans relance téléphonique. Notre étude montre une bonne précision du géocodage rétrospectif des adresses des sujets depuis leur naissance. Il s'agit d'une condition importante pour caractériser les expositions environnementales sur plusieurs décennies par le biais d'une approche SIG. Du fait de la mobilité des sujets, du manque potentiel de puissance et des variations locales de l'incidence, la réalisation d'une étude interrégionale, voire nationale, apparaît préférable. ▼

Remerciements. Les auteurs remercient Marine Genton, doctorante au CLB, pour son aide dans le cadre de la présente étude. Les auteurs remercient également les volontaires ayant participé à l'étude et l'équipe de la maternité de la Croix-Rousse, à Lyon. Le projet TESTEPERA a reçu un financement du Cancéropôle Lyon Auvergne Rhône-Alpes (CLARA), de la Fondation de France, et un financement conjoint de l'Institut national du cancer (INCa) et de l'Agence française de sécurité sanitaire de l'environnement et du travail (AFSSET). Rémi Béranger est titulaire d'une allocation doctorale de recherche attribué par la Région Rhône-Alpes.

Liens d'intérêts : les auteurs déclarent n'avoir aucun lien d'intérêt en rapport avec cet article.

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IV.3 (article #6)

Studying the impact of early life exposures to pesticides on the risk of testicular germ cell tumors during adulthood (TESTIS project): study protocol

Rémi Béranger[1,2,3], Olivia Pérol[1], Louis Bujan[4,5], Elodie Faure[1], Jeffrey Blain[1], Charlotte Le Cornet[1,2], Aude Flechon[6], Barbara Charbotel[7], Thierry Philip[1], Joachim Schüz[2], Béatrice Fervers[1,3]

(1) Unité Cancer et Environnement, Centre Léon Bérard, Lyon, France

(2) Section of Environment and Radiation, International Agency for Research on Cancer (IARC), Lyon, France

(3) EAM 4128 "Santé Individu Société", Université Claude Bernard – Lyon1, Villeurbanne, France

(4) CECOS, Hôpital Paule de Viguier; Fédération Française des CECOS, CHU Toulouse, France

(5) Université de Toulouse; UPS; Groupe de recherche en Fertilité Humaine (EA 3694, Human Fertility Research Group), Toulouse, France

(6) Centre de Lutte Contre le Cancer, Centre Léon Bérard, Lyon, France

(7) Université de Lyon, F-69003 Lyon, France; Université Lyon 1, UMRESTTE (Unité mixte IFSTTAR/UCBL), Domaine Rockefeller, 69373 Lyon, France

Article published in *BMC cancer*
(2014; 14:563)

Béranger et al. BMC Cancer 2014, 14:563
http://www.biomedcentral.com/1471-2407/14/563

BMC Cancer

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Studying the impact of early life exposures to pesticides on the risk of testicular germ cell tumors during adulthood (TESTIS project): study protocol

Rémi Béranger[1,2,3*], Olivia Pérol[1], Louis Bujan[4,5], Elodie Faure[1], Jeffrey Blain[1], Charlotte Le Cornet[1,2], Aude Flechon[6], Barbara Charbotel[7,8], Thierry Philip[1], Joachim Schüz[2] and Béatrice Fervers[1,3]

Abstract

Background: The incidence of testicular germ cell tumors (TGCT), the most common cancer in men aged 15 to 45 years, has doubled over the last 30 years in developed countries. Reasons remain unclear but a role of environmental factors, especially during critical periods of development, is strongly suspected. Reliable data on environmental exposure during this critical time period are sparse. Little is known on whether it could be a combined effect of early and later-life exposures.

Methods/Design: Our research aims to study the association between TGCT risk and pesticide exposures (domestic, occupational and environmental) during critical time periods of development and combined early and later-life exposures. The study design, developed during a 2-year pilot study, is a multicenter case–control study of 500 cases (ascertained through histology) and 1000 fertile/fecund controls recruited through 21 French '*Centres d'Etude et de Conservation des Œufs et de Sperme humain*' (CECOS). Trained professional interviewers interview the subjects and their mothers by phone. Using a geographic information system developed and tested for application in this study design, environmental pesticides exposure assessment is based on life-time residential history. Occupational pesticides exposures are assessed by an industrial hygienist based on parents' occupations and tasks. Exposures during the prenatal period, early childhood and puberty are focused. A blood sample is collected from each participant to assess genetic polymorphisms known to be associated with TGCT risk, as well as to explore gene-environment interactions.

Discussion: The results of our study will contribute to better understanding the causes of TGCT and the rapid increase of its incidence. We explore the effect of combined early and later-life pesticides exposure from multiple sources, as well as potential gene-environment interactions that have until now been rarely studied for TGCT. Our design allows future pooled studies and the bio-bank allows additional genetic or toxicological analyses.

Keywords: Case–control studies, Pesticides, Maternal exposure, Paternal exposure, Geographic information systems, Testicular neoplasms, Germinoma, Environmental exposure, Occupational exposures, Gene-environment interaction

Background

Testicular Germ Cell Tumors (TGCT, testicular cancer) represent the most frequent cancer in young men aged 15 to 45 years in developed countries with primarily Caucasian populations. TGCT incidence has been increasing throughout Europe over the last 30 years, including in France, where the annual incidence rate has doubled from 3.4/100 000 in 1980 to 7/100 000 in 2008 [1-3]. Large geographical variation in incidence rates exists between different European countries with West–east and North–south gradients [2,4]. The reasons for such a phenomenon are still unclear but a role of environmental factors is strongly suspected. The rapid increase of TGCT incidence rates and the evolution of the incidence rate in migrant populations [5,6] support this hypothesis. However, TGCT risk varies also by ethnicity (Caucasian men have a higher TGCT risk than men in Asian or African

* Correspondence: r.beranger26@gmail.com
[1]Unité Cancer et Environnement, Centre Léon Bérard, 28 rue Laennec, 69373 Lyon, 08 Cedex, France
[2]Section of Environment and Radiation, International Agency for Research on Cancer (IARC), Lyon, France
Full list of author information is available at the end of the article

populations) [7], and familial history of TGCT is also known to be associated with an increased TGCT risk [8], supporting a potential role of genetic factors. It is estimated that 13% of TGCT have a genetic origin [8]. Individual factors have also been suggested to be associated with TGCT risk [9,10] and several studies have suggested a positive association between a higher socioeconomic status and TGCT occurrence [11-13], although this relationship was not consistently found [14].

Given the peak incidence of TGCT in very young adults and the fact that TGCT has been shown to develop through carcinoma-in situ cells of fetal origin [15], the role of early exposures, in particular during the critical time windows when the reproductive tract develops has been hypothesized [16,17]. The concept of the Testicular Dysgenesis Syndrome (TDS) proposes that an impaired development of fetal testes may lead to an increased risk of cryptorchidism, hypospadias, testicular cancer and decreased spermatogenesis [17,18]. However, the TDS incidence in the general population is unknown and to what extent these disorders are actually biologically related through a fetal mechanism remains unresolved. Although the concept of TDS remains controversial [19,20], the hypothesis of a pre-natal origin of TGCT and a role of in-utero or early childhood exposures to environmental factors in TGCT development remain widely accepted. A combined effect of prenatal, early and later-life (adolescence or adulthood) exposures has also been suggested [21], but has not been explored so far.

It is generally accepted that the development of TGCT is under endocrine control and exposures to chemicals with endocrine disrupting properties, including pesticides, have been suggested to be associated with an increased TGCT risk in epidemiological studies [21,22]. So far, no appropriate animal models for TGCT exist, therefore our knowledge on factors involved in TGCT development is based on epidemiological research [23]. Reliable data on occupational or environmental risk factors during adulthood are sparse, and only a few studies have investigated parental exposures as a contributing factor to the risk of testicular cancer occurring 20–40 years later [24,25]. Available studies were often limited by small sample size or by too broad exposure assessment. Furthermore, genetic polymorphisms might be involved in gene-environment interactions by increasing the susceptibility to the effect of endocrine disruptors [26], but these were rarely considered. Additional studies have investigated the place of residence (urban versus rural location), as a surrogate for pesticides exposure, but showed inconsistent results and none of these included the subject's or parental residential history, or any detailed assessment to environmental pesticides exposure [27-30].

Accurate characterization of environmental pesticides exposure, especially in retrospective studies, is often difficult due to the lack of exposure data available at the individual level, and due to the inherent limitations of conventional epidemiological methods for this type of studies (e.g. recalls bias due to self-reported information). However, some studies have found a positive association between residential proximity to cultivated agricultural fields and pesticides concentrations in biological samples of residents [31-33]. Based on these observations, Geographic information systems (GIS) offer the opportunity to retrospectively assess agricultural pesticides exposures by collecting and analyzing historical environmental data over large areas [34,35]. To our knowledge, GIS technology has not been applied to study environmental pesticide exposures in relation to TGCT risk.

The discordant findings and the limitations of available studies concerning TGCT risk factors underline the importance to conduct studies with sufficient statistical power to detect risks associated with exposures during critical windows of vulnerability, as well as combined perinatal and later life exposures. The current evidence further underscores the need for studies that can accurately characterize pesticides exposures from multiple sources (environmental, occupational and domestic). Therefore, we conducted a two year pilot study to develop a study design for the case–control study presented here, to compare the effectiveness of different approaches for cases', controls' and mothers' recruitment in the French context and to verify our ability to collect relevant data on exposures during subjects' perinatal period (TESTEPERA project) [36]. Based on this pilot study, we chose a national, prospective, face-to-face recruitment. The results also confirmed our capacity to reconstruct cases' and controls' occupational and residential history and to accurately geocode most of the addresses (82%), including for early life periods.

Objectives

Our case–control study aims to assess the impact of multisource pesticides exposure (domestic, occupational and environmental) during prenatal and early childhood periods on the risk to develop a TGCT during adulthood. The study is also designed to assess potential gene-environment interactions as well as the hypotheses of combined prenatal and later life exposure.

Methods/Design

Study design

The TESTIS study is a national, multicenter, prospective case–control study of 500 cases and 1000 controls (two groups of 500). Cases are recruited prospectively through the French centers for semen conservation (*Centres d'étude et de conservation d'oeufs et de sperme humain*, CECOS). Controls are recruited in CECOS and centers of assisted reproduction (group A), and regional maternities

contiguous to the CECOS centers (group B). Twenty-one out of the 23 French CECOS, included in a national network (*Fédération Française des CECOS*), agreed to participate to the study (Besançon, Bordeaux, Caen, Clermont-Ferrand, Dijon, Grenoble, Lille, Lyon, Marseille, Montpellier, Nancy, Nice, Paris Tenon, Paris Jean Verdier, Paris Cochin, Reims, Rennes, Rouen, Strasbourg, Toulouse, Tours). The study protocol was approved by the French regulatory authorities and by the appropriate French ethics committees (*Comité consultatif sur le traitement de l'information en matière de recherche dans le domaine de la santé (CCTIRS)*; *Comité de protection des personnes (CPP)*; *Agence nationale de sécurité du médicament et des produit de santé (ANSM)*). The study was registered at http://www.clinicaltrials.gov (NCT number: NCT02109926). Figure 1 shows the organization of the study for recruitment, data collection, biological sampling and samples conservation.

Study population

The study population consists of men aged 18 to 44 years at date of diagnosis, born in metropolitan France, with a valid health insurance affiliation. Cases are men diagnosed with seminoma or non-seminoma TGCT (ascertained through histological report), and seen in one of the participating CECOS for sperm cryopreservation. Controls of group A are sperm donors or partners of infertile women having a normal sperm count (>39 million per ejaculate [37]). Controls of group B are partners of pregnant women hospitalized for a pathological pregnancy in the level III maternity (regional maternity) adjacent to the CECOS. Participants have to sign an informed consent form. Subjects unable to write and understand French language, as well as subjects presenting severe psychological or mental disorders, and subjects under legal guardianship are excluded. Controls with a history of cryptorchidism or of TGCT are also excluded. Two controls (one of each group) are matched to each case on age (±2 years) and the recruiting center.

Considering the incidence of TGCT and the CECOS activity, we estimate a 18-month duration to recruit all cases and controls. The recruitment is performed by physicians and/or midwives (investigators). A written permission is asked to cases and controls to contact their mothers (or the closest relative alive, if the mother is deceased or cannot be interviewed). Participants who sign the informed consent receive a document to prepare information prior to the phone interview, including: lifetime residential history, addresses of schools during childhood, parental occupation at subjects' conception, birth information, and job history. Investigators propose to each case and control to participate in a blood sample collection. Information on subjects is recorded using a protected on-line study platform that generates a unique identification number for each participant. Cases and controls receive a financial compensation for their participation: 20 euros for completion of the interview and 40 euros for participation in both blood sampling and completion of the interview.

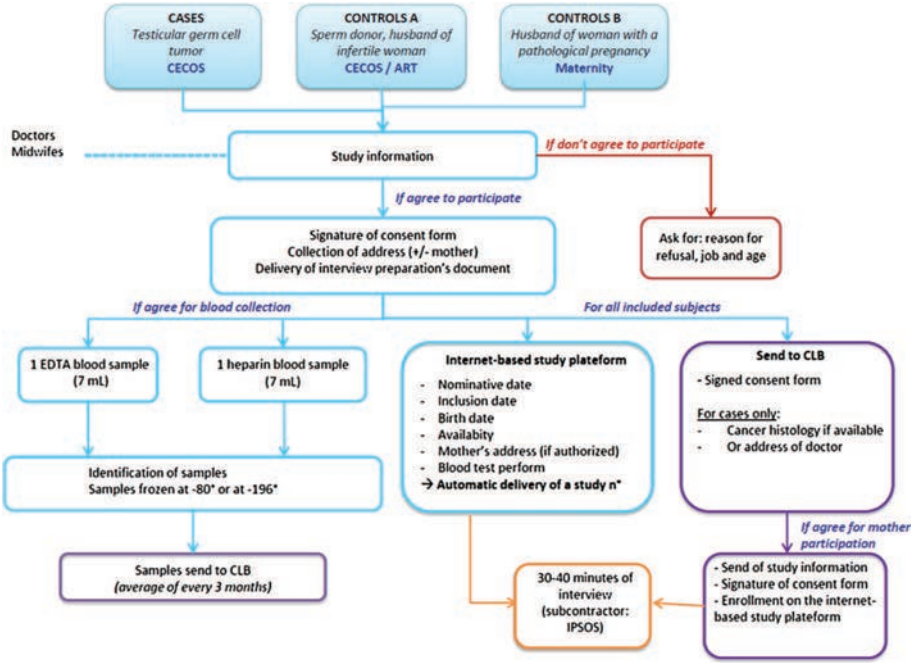

Figure 1 Schematic organization of the recruitment and the data collection. ART, Center for Assisted reproductive technology; CECOS, French center for semen conservation; CLB, Centre Léon Bérard; EDTA, Ethylene diamine tetra-acetic acid.

The Principal investigator (PI) contacts case and control mothers (or closest relative alive) upon written permission of cases and controls. Subjects are asked to inform their mother/relative beforehand. Mails include an information letter, a consent form (to be returned using an enclosed pre-paid envelope) and a document to prepare information on residential and job history prior to the interview. Mothers are registered on the same internet platform after the reception of the signed consent form. In absence of any response, up to three phone reminders are done, at two-week intervals.

Biological sampling and storage

For each case and control that agrees to participate to the blood sampling, two samples are collected at the inclusion by the investigators (1 × 7 ml EDTA (Ethylene diamine tetra-acetic acid) tube, 1 × 7 ml heparinized tube). Blood samples have to be centrifuged within one hour from sampling. For CECOS having −80°C storage, EDTA tube might be stored directly. For CECOS having −196°C storage only (all CECOS are equipped for sperm and egg conservation), buffy coat have to be extracted from the EDTA tube and stored in an adapted cryotube. Concerning the heparinized tube, plasma have to be extracted and stored in 1 ml aliquot in cryotubes at −80°C or −196°C depending on the equipment of each CECOS. Cryotubes are identified using the personal identification number attributed by the online study platform to each participant. Samples are gathered and shipped regularly using a specialized transporter to the Biological Resources Center (BRC) of Centre Léon Bérard (CLB), Lyon. At the BRC, DNA is extracted from the buffy coat (in case of −196°C preservation) or the EDTA tube (in case of −80°C preservation) using the AUTOPURE automaton (Quiagen, Germany). Then, DNA is stored at −80°C (300 ng DNA per aliquot).

Data collection

Data from cases, controls and case/control mothers (or relative) are collected through a standardized phone questionnaire administrated by professional interviewers (IPSOS company). Interviewers are unaware of the case or control status of study subjects. To ensure consistency in data collection, interviewers have been trained in the completion of the questionnaire and provided with a field guide. All data are entered directly on the specially designed online study platform used for registration and data collection (for items, see Table 1). Investigators, technicians and researchers have a personal login and password to access the platform. Accesses to sensitive data are restricted, based on the user profile. The coordinating center contact the clinicians in charge of cases to obtain the pathology report and serum markers (alpha feto-protein, beta-HCG). For eligible subjects who refuse to participate, age, job, and reason for refusal are collected by investigators and entered into the study platform.

Occupational exposure assessment

Occupational exposure assessment of prenatal and early postnatal periods involve encoding parental occupations (mother: from the beginning of the job history to the 17 years old of the subject; father: from one year before conception to the 17 years of the subject). Based on the occupational history and job/task related information, all occupations are encoded by an industrial hygienist according to the International Standard Classification of Occupations (ISCO). The ISCO-68 is used to classify jobs of subject's parents, and the ISCO-08 is used for the subject's jobs. The French nomenclature of activity (NAF) is used to code the industrial classification of all jobs. In a second step, an industrial hygienist performs a detailed occupational exposure assessment based on job and task descriptions. Specific items have been added to the questionnaire to help the hygienist to assess exposures suspected to be associated with TGCT: pesticides, plasticizers, solvents, welding fumes and heavy metals. For each job held, probability, intensity (low, intermediate, strong) and duration of exposure are encoded.

Domestic exposure assessment

Domestic exposure to pesticides is assessed for cases, controls and their mothers. Specific items in the questionnaire cover the main domestic pesticide use by interviewees and persons sharing the same household (gardening, pet treatment, indoor usage of insecticides or fungicides, and lice treatment), as well as the frequency of use. Pesticide exposure (compound family, probability of exposure and intensity) are estimated through expert assessment, based on the pesticide-use matrix developed by the National Cancer Institute (MD, US) [38].

Environmental exposure assessment

Cases and controls residential history are gathered from 1 year prior to birth to date of the inclusion in the study. Semi-automatized fields in the study platform help to reduce misspellings when entering the questionnaire data. In case of inconsistency between subjects' and mothers' information, data provided by the mothers are used. Addresses are geocoded using the database "*BD adresse*" from the French National Geographic Institute (IGN), which contains coordinates of all addresses in France (unit: RGF Lambert 93). Since the coordinates of the database are centered on the postal address, a GIS technician moves manually the point to the center of the household. Using dedicated software (BD Adresse® for ArcGIS Locator), we identify all addresses geocoded with poor precision (at the street level or less) for manual verification or repositioning (when possible). Specific additional questions are added to

Table 1 Items collected during the phone interview

Categories	Cases and controls	Mothers (or closest relatives)
General information	Medical history and long term treatments (childhood); Birth characteristics; Geographical origin; Socio-economic status	Medical history (mother); Treatments during pregnancy; Age and morphology at birth; Birth characteristics (son); Socio-economic status
Occupational exposures	Entire job history (+tasks and company name and addresses); Specific questions on pesticides, solvents, welding fumes, heavy metals and plastic exposures	Job history from the beginning to the 17 years of the son (mother)/job history from 1 year before the conception to the 17 years of the son (father); Specific questions on pesticides, solvents, welding fumes, heavy metals and plastic exposures
Environmental exposures	Whole residential history and households characteristics; Addresses of schools	Residential history from 1 year before son's conception to the 17 years of the son
Domestic exposures	Domestic use of pesticides gardening, pet treatment, indoor usage of insecticides or fungicides, and lice treatment (at puberty)	Domestic use of pesticides gardening, pet treatment, indoor usage of insecticides or fungicides, and lice treatment (son: perinatal period and at puberty)
Lifestyle	Smoking status; Drug use; Physical activity	Smoking status; Drug use

the questionnaire to help the GIS technician when no street number is available (closest crossing road or point of interest).

The use of infra-red images, based on the greenness reflectance, has been used successfully in previous US studies to reconstruct land use data [39,40]. Satellites images are available from the Landsat® program from 1972 with an 80 m spatial resolution and from the SPOT® program from 1986 with a 20 m spatial resolution. Based on remote sensing of satellite images and/or photo interpretation of IGN aerial photography (available from 1920's), we determine the land use around each residence of interest. When data are not available or of poor quality, we assign land use data of the nearest available time period to the residence. Public data from agricultural statistics (Recensement Statistique Agricole, DRAAF) as well as expert assessment are used to validate our land use layer, when needed. The agricultural statistics provide the proportion of each type of crops in each municipality for 1970, 1979, 1988, 2000, and 2010.

The GIS based approach has been developed in a previous study (SIGEXPO project [41]). We investigated the link between environmental parameters (crop acreage, characteristics of neighboring cultivated fields, geographic and meteorological variables) and the concentration of pesticides in indoor dust of nearby homes. More than 700 samples were collected from 239 volunteer homes in the Rhône-Alpes region, France. These were distributed according to the different types of territories and according to different levels of intensity of theoretical exposure. Samples were taken during the main period of pesticides use according to the representative cultures of the Rhône-Alpes region (orchards, wine, cereals), reflecting the main application modes used in France (rotary atomizer, inflatable ramps, pneumatic sprayer, and motorized mist blower mounted on straddle tractors). According to this study, our GIS methodology is based on 500 meter and 1000 meters buffers, the frequency of the wind direction (data from Meteo France®), the presence of topographic barriers (BD Alti, IGN), vegetative barriers (BD Topo®, aerial photography and/or remote sensing), and structural barriers (BD Topo®, aerial photography and/or remote sensing). The score we developed (Agricultural Exposure Index (AEI)) estimates the intensity of exposure to the different crop types for each address/year.

Specific attention is given to locations during known or suspected critical lifetime periods in the etiology of TGCT (prenatal, early life, puberty). The AEI can be used as a surrogate of agricultural pesticide exposure level. In a second step, we use pesticides matrices to convert the crop exposure level into a pesticide exposure level, for each family of pesticides (or compound per compound, when available). These matrices contain the list of pesticides likely to have been used depending on the type of crop and period. Two matrices are currently under construction in France and might be used in our study: MATPHYTO (*Institut de Veille Sanitaire, France*) and PESTIMAT (*Institut de Santé Publique, d'Épidémiologie et de Développement, France*).

Social deprivation and territorial indicators

To determine the impact of social deprivation on TGCT risk, using the Townsend index [42] and European Deprivation Index (EDI) [43], individual and territorial socio-economic data are collected to be included in the GIS. Territorial data are available from INSEE (French National Institute for Statistics and Economic Studies) at the IRIS scale (acronym for 'aggregated units for statistical

information', covering a target size of 2000 residents per basic unit).

Genetic analyses

We investigate polymorphisms known to be associated with TGCT risk. We identified 45 Single Nucleotide Polymorphisms (SNPs) revealed through 4 Genome Wide Association Studies and one replication study (from 8 loci: KITL, BAK1, SPRY4, ATF7IP, TERT, DMRT1, TGFBR3 and BMP7) [8,44-47]. Associated odds ratios (OR) were 1.37 (95%IC 1.1 – 1.58, p = 10 e-13) for polymorphisms on chromosome 5; 1.50 (95%IC 1.28 – 1.75, p = 10 e-13) for polymorphisms on chromosome 6; and 2.55 (95%IC 2.05-3.19, p = 10 e-31) for polymorphisms on chromosome 12. Additionally, we identified 6 additional SNPs associated with organochlorine metabolism pathways and known to be able to modify the risk to develop a TGCT (2 loci: CYP1A1 and HSD17B4) [26]. Other polymorphisms may be added if new publications suggest additional polymorphisms prior to the genetic analysis. Considering rapidly decreasing costs of genetic analyses, a genome wide screening might become an alternative option for SNPs analyses.

Determination of the sample size

Our research will examine the association between TGCT risk and occupational, domestic and environmental pesticides exposure. Since occupational pesticides exposure is supposed to be less frequent and associated with higher exposure levels than domestic and environmental exposures, we based our sample size calculation on the prevalence of occupational pesticides exposure among case and control parents. Based on the prevalence of agricultural workers in France in 1988 (10%) (http://agreste.agriculture.gouv.fr/IMG/pdf/AGRIFRA07c-2.pdf), the minimum detectable odds ratio with our sample (500 cases and 1000 matched controls) is 1.6, considering a statistical power of 80% at a significance level of 5%. Considering additional occupations associated with pesticides exposure (e.g.: greenhouse worker, sawmill worker, forester), the total prevalence of exposed workers should be higher. Considering a prevalence of exposure of 15%, the minimum detectable odds ratio under the conditions outlined above is 1.5.

Statistical analyses

Standard descriptive statistics will be used to describe characteristics and pesticide exposure of cases, controls and mothers/relatives. Exposure variables will be explored using supervised principal component analyses [48]. Risk analyses will be based on conditional logistic regression models, to compute odds ratios for TGCT at different levels of exposure. Exposure variables will be investigated as continuous variables (with appropriate transformation to achieve normality) as well as categorical variables (quartiles or predefined categories depending on variable type). In addition we will create a combined pesticide exposure variable based on pesticide exposure (occupational, environmental and domestic) during prenatal and early postnatal period (PEPPP) and during adolescence (PEA). We will examine PEPPP exposure, PEA exposure, and combined PEPPP and PEA exposure in relation to TGCT risk. The effects of additional potential confounders (other than our matching criteria) on the associations between pesticide exposure and risk of TGCT will be examined and added to the model one by one. Comparison between models with and without adjustment will be used to examine the potential confounding effect of these factors and only factors with relevant changes in the odds ratios will be kept in the final model. Potential confounders include the geographical origin, socioeconomic status, tobacco and cannabis consumption, length and weight at birth, birth order, and the familial history of TGCT.

The two control groups will be used first as separate control groups to identify potential major differences. In order to examine whether any of the associations between pesticide exposures and TGCT risk differ by subgroups of known risk factors or by genetic polymorphisms, we will perform additional stratified analyses. Tests for statistical interaction will be used to examine whether any apparent heterogeneity of effect is statistically significant. This will be done by comparing models with and without interaction terms between the risk factor or genetic polymorphisms and the environmental variable (pesticide exposure) with a maximum likelihood ratio test. Sensitivity analyses are planned to explore the impact of potential bias or methodology limitations (such as quality of satellite images, through removing subject born before 1986 having less precise satellite images at time of birth).

Steering committee

A Steering Committee has been implemented to oversee the study progress. It is composed of the two principal investigators, a project manager, a doctoral student, study partners, two oncologists specialized in TGCT and eight CECOS representatives. The Steering Committee will meet every 4 months. It will be regularly informed about study progress and of any emerging problems. It will monitor compliance with the study protocol, the quality of collected data and will review scientific reports and publications.

Discussion

Considering the rarity and latency of TGCT, the case–control design appears to be the most appropriate method for our research. Based on a two-year pilot

study, the TESTIS project was optimized to address short comings identified by previous research despite the rarity of the TGCT. In France, around 2000 men are diagnosed with TGCT each year and at least 1100 TGCT patients are seen annually for sperm cryopreservation in the CECOS network. The CECOS have a regional recruitment, and only the CECOS are allowed to cryopreserve sperm in France. Based on our pilot study, loss due to inclusion/exclusion criteria and to non-participation should not exceed 50%. Thus, we estimate that 18 months is long enough to ensure the recruitment of 500 TGCT cases in the 21 participating CECOS.

Testicular cancer and reproduction are sensitive topics. Recruiting the controls through hospital settings aims to facilitate the recruitment of our young male population, as well as managing biological sampling. To ensure that both cases and controls are recruited at a regional level, we decided to select Group B controls among partners of women having a pathological pregnancy. The latter are managed centrally in the regional maternities adjacent to the participating CECOS, while selecting controls among partners of all pregnant women in these maternities would lead to over-representation of the urban population. With a participation of 21 out of 23 CECOS, we assume that our sample is representative to the French metropolitan territory.

In general, young men are difficult to approach and less likely to participate to research than other population groups. Low response rates make difficult ascertaining a population-based control group representative of the general population. Since no perfect control group was found, we choose two distinct control groups to test our hypotheses on populations presenting different aspects of the general population, as made by Stang et al. [49]. Controls from Group A and Group B present both advantages and weaknesses (see Table 2). Associations found consistently in the two control groups separately will strengthen our hypotheses, whereas inconsistent findings will provide new insights on potential confounding factors.

According to the TDS hypotheses, TGCT, cryptorchidism, hypospadias and several forms of male infertility are suspected to share common etiological factors [17,18]. By choosing controls supposed to be fecund or having a normal sperm count, we aim to avoid subjects suffering from a minor form of TDS. In consequence, our controls are likely to be more fertile than the general population of the same age, and may present lower exposure to reproductive toxicants during postnatal periods. This should be taken into account in the interpretation of results when considering adolescent or adulthood exposure. Also, setting up a third control group, more representative of the general population (e.g. by selecting healthy young males from an existing cohort study conducted in France), may be considered.

The financial compensation for cases and controls participation will help recruitment, although the amount of compensation is kept low so subjects are not tempted to participate in the research against their personal convictions. Minimal information (age, occupation and reason for refusal) will be collected from subjects refusing participation to compare participants and non-participants and identify differential non-participation. Additionally, our study population may be compared to the general population based on data from the French National Institute for Statistic and Economic Studies (INSEE). This step will require coding all jobs according to the French classification of jobs and socio-economic categories from 2003 (PCS-2003).

Since prenatal information is of major interest to our research, we decided to include mothers of cases and controls in our study. Previous studies shown that mothers' participation rate range from 54% to 71% [50,51], which was similar to our pilot study [36]. Minimum information regarding parental occupation and residential addresses

Table 2 Main advantages and weaknesses of the two control groups

	Advantages	Weaknesses
Control Group A *(sperm donators & fertile partners of infertile woman)*	- Direct access to the subject (face to face recruitment & blood sampling)	- Older than cases/difficult to recruit subjects below 25 years old
	- More concerned by the topic/good participation rate	- Live with infertile woman/more exposed to reproductive toxicant than general population?
	- Sperm count available	
	- Regional recruitment	
Control Group B *(partners of pregnant woman hospitalized for pathological pregnancy)*	- Same age group than cases	- More difficult to approach (visit during evening/week-end)
	- Direct access to the subject (face to face recruitment & blood sampling)	
	- Presumably fecund	- No available serologies (need to store blood samples in separate areas)
	- Regional recruitment	- Link between subjects' exposures partners' pathological pregnancy?
	- Large population/easy to match with cases	

during the prenatal period is included in the case and control questionnaire to reduce missing data in case of mother's non participation. Reliability of these items will be assessed by comparing information collected from participating case and control mothers and their respective sons.

Our biological sample collection will allow further ancillary projects. Heparinized plasma may be used to search for organic pollutants (multiresidue analyses) or biomarkers, whereas DNA samples will allow participating in genome wide association studies or perform DNA methylation studies. Biological samples will be made at the time of diagnosis, before any radio- or chemo-therapy (information recorded at time of the inclusion).

To reduce recall bias and to minimize missing data, we use objective criteria for exposure assessment when possible (job history analyzed by an industrial hygienist; use of GIS methods based on residential history). All subjects receive a document to gather information on residential and job history prior to the interview. The use of trained interviewers, blinded to the case or control status will ensure the same degree of questioning for both cases and controls.

A risk of misclassification for the GIS method remains due to imprecision in geocoding of residential addresses. Our pilot study allowed precise geocoding for 82% of all subjects' residences. Lower precision was significantly associated with size of communes (<10,000 inhabitant), mainly related to rural addresses or hamlet lacking street numbers. No statistical difference in accuracy was found according to time period. In a recent French study, Semalgue-Faure et al. estimated that the medium imprecision of addresses when placed at the center of the hamlet or the street was about 200 meters [52]. Our geocoding accuracy was similar to this study [52], but slightly lower compared to several US studies [53-55]. However, geocoding of US addresses showed decreasing precision for older addresses [53]. Yet, imprecision does not lead to differential misclassifications. To improve ascertainment of the most accurate addresses, instructions have been given to interviewers on how to obtain complete addresses, or if not available, request ancillary information such as names of nearest intersecting roads or a nearby landmark still likely to be in place.

We expect our results to contribute to better understanding of the causes of TGCT and the rapid increase of its incidence. Thanks to the interdisciplinary network of research teams, we will be able to explore the effect of combined early and later-life pesticides exposure from multiple sources, as well as potential gene-environment interaction that have been poorly studied for TGCT. The use of GIS to assess environmental exposure to agricultural pesticide as well as the combination of environmental, domestic and occupational exposure are innovative and will improve exposure characterization. The thorough geocoding of subjects' lifetime residential history will allow analyses of additional environmental risk factors in future studies as new hypothesis emerge. This research is an innovative approach in France that will contribute to improve our knowledge on the long term effects of pesticide exposure on human development, and potentially provide support for decisions in future healthcare policies.

Competing interests

Authors declared no competing interest.

Authors' contributions

Drafting the study protocol: RB and OP (supervision: JS, BF, and TP); Strategy for subject's recruitment: LB; Methods for environmental exposures assessment: RB, EF, and JB; Strategies for occupational exposures assessment: BC and CLC; Expertise on TGCT: AF; Statistical analyses: RB, BF and JS; All authors have contributed to the writing of the present manuscript. All authors have read and approved the final manuscript.

Acknowledgments

The authors acknowledge the members of the TESTIS Steering Committee (Dr. Béatrice Fervers, Dr. Joachim Schüz, Pr. Louis Bujan, Dr. Barbara Charbotel, Dr. David Cox, Dr. Virginie Chasles, Dr. Aude Flechon, Dr. Helen Boyle, Olivia Pérol, Rémi Béranger, Dr. Florence Brugnon, Dr. Florence Eustache, Dr. Isabelle Koscinski, Dr. Jeanne Perrin, Pr. Célia Ravel, Pr. Nathalie Rives, Dr. Sandrine Giscard d'Estaing, Dr Véronique Drouineaud). The authors also thank Gilles Clapisson (Centre Léon Bérard, Lyon) and Pr. John R. Nuckols (Colorado State University, CO, US) for their support in the design of the study protocol. Rémi Béranger received a doctoral grant from the Rhône-Alpes region. This project is granted by the French National Institute of Health and Medical Research (INSERM, N°ENV201306/CLB) and the French National Cancer Institute (INCa, N°2013-143).

Author details

[1]Unité Cancer et Environnement, Centre Léon Bérard, 28 rue Laennec, 69373 Lyon, 08 Cedex, France. [2]Section of Environment and Radiation, International Agency for Research on Cancer (IARC), Lyon, France. [3]EAM 4128 "Santé Individu Société", Université Claude Bernard – Lyon 1, Villeurbanne, France. [4]CECOS, Hôpital Paule de Viguier; Fédération Française des CECOS, CHU, Toulouse, France. [5]Université de Toulouse; UPS; Groupe de recherche en Fertilité Humaine (EA 3694, Human Fertility Research Group), Toulouse, France. [6]Centre de Lutte Contre le Cancer, Centre Léon Bérard, Lyon, France. [7]Université de Lyon, F-69003 Lyon, France. [8]Université Lyon 1, UMRESTTE (Unité mixte IFSTTAR/UCBL), Domaine Rockefeller, 69373 Lyon, France.

Received: 23 June 2014 Accepted: 22 July 2014
Published: 4 August 2014

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doi:10.1186/1471-2407-14-563
Cite this article as: Béranger *et al.*: Studying the impact of early life exposures to pesticides on the risk of testicular germ cell tumors during adulthood (TESTIS project): study protocol. *BMC Cancer* 2014 **14**:563.

Chapter V:
General discussion

V.1 Synthèse en français / summary in English

RESUME - FRANCAIS

Notre revue de la littérature (objectif n°1) a confirmé le manque de données autour de l'impact des expositions prénatales aux pesticides sur le risque de TGTC, ainsi que le besoin d'approches plus fiable pour estimer les expositions environnementales aux pesticides. Dans la mesure où les données de la littérature ne permettaient pas de développer une approche GIS pour la France dans ce cadre, nous avons cherché à identifier les déterminants environnementaux de l'exposition domestique aux pesticides agricoles (2ème objectif de la thèse). A partir de ces résultats, une approche GIS sera développée dans les prochains mois, mais elle nécessitera un nouveau jeu de données pour sa validation. L'étude pilote (3eme objectif) confirme notre capacité à recruter des hommes jeunes et leur mères, à collecter des informations concernant leurs expositions potentielles, et à géocoder précisément leurs adresses jusque dans les années 70. A partir de ces objectifs intermédiaires, nous avons pu optimiser et finaliser le protocole de l'étude TESTIS, conformément à l'objectif principal de la thèse.

Pour la première fois, les données présentées dans la thèse montrent que la direction des vents ainsi que les barrières végétales sont des déterminants potentiels de la contamination des poussières domestiques par les pesticides agricoles. Ces résultats sont cohérents avec les hypothèses préexistantes et les études sur les brise-vents préalablement menées. Ils sont également consolidés par notre étude de validation montrant la bonne efficacité de notre lingette en cellulose concernant la collecte de pesticides. Nos mesures ont également suggéré que l'utilisation domestique de pesticides est une source majeure de l'exposition intérieure aux pesticides, sur la base d'un nombre important de pesticides analysés. Ces résultats suggèrent que les études se basant sur les GIS et négligeant les expositions domestiques sont exposées à des biais de classement susceptible de diluer l'association observée. Ces résultats nous ont conduits à inclure les expositions domestiques dans le projet TESTIS.

Il reste toutefois certaines limites à résoudre. Si les groupes témoins du projet TESTIS ont été choisis pour permettre d'explorer au mieux l'impact des expositions prénatales, ils ne sont pas adaptés à l'exploration des expositions environnementales de l'adulte. Des stratégies devront

également être développées au sein de l'unité pour permettre de reconstituer les données d'occupation des sols jusque dans les années 70, de manière à permettre l'utilisation de GIS sur cette période. Enfin, d'autres études sont nécessaires pour vérifier la corrélation entre la contamination des poussières domestiques et la contamination sanguine ou urinaire des habitants.

Pour conclure, nous résultats ont permis le développement du projet TESTIS, qui a été financé et qui est en cours de réalisation. Ce projet aidera à mieux comprendre l'impact des expositions prénatales aux pesticides sur le risque de développer un TGCT. Cette thèse fourni également des informations clés et novatrices concernant l'exposition domestique des ménages français, les déterminants de l'exposition des ménages aux pesticides agricoles ou encore les facteurs susceptibles d'influencer l'efficacité de certaines méthodes de prélèvement pour les pesticides. En plus du projet TESTIS, cette thèse sert de base à plusieurs autres projets multidisciplinaires en cours de lancement ou de développement.

SUMMARY - ENGLISH

Our literature review (1st objective) confirmed the gap in knowledge regarding the association of prenatal pesticide exposures and TGCT risk, and highlighted the need for more reliable approaches to assess environmental pesticide exposures. Because data available from the literature did not allow us to develop a new GIS metric for France, the 2nd objective of the thesis was to identify the environmental determinants of exposure to agricultural pesticides. Based on our findings, a GIS metric will be developed in the near future, but its validation will require additional samples. The pilot study (3rd objective) confirmed our ability to recruit young men and their mothers, to collect information about their potential exposures and to geocode their addresses going back to the 1970's. Based on these results and the findings of the SIGEXPO study, the protocol of the TESTIS case-control study was optimized and finalized, which was the main objective of the thesis.

For the first time, results from this thesis showed that prevailing winds and vegetative barriers are potential determinants of the indoor dust pesticide contaminations. These findings were in line with previous theories and with methodological studies of wind-breaker. The overall good

pesticide collection efficiency of our cellulose wipes strengthens these findings. Our measurements also identified domestic use as a major source of indoor pesticide exposure, based on a large number of pesticides screened. These novel findings suggest that GIS-based studies neglecting domestic pesticide use may suffer from exposure misclassification, potentially diluting any association. These results led us to consider domestic pesticide exposure assessment in the TESTIS study.

However, some issues remain to be solved. While control groups of the TESTIS study have been chosen to explore prenatal risk factors, it may not be optimal to explore adult risk factors. Further work should be done to define the strategy to reconstruct land use until the 1970's in order to implement the GIS to assess environmental pesticide exposures. Furthermore, additional analyses are needed to assess the correlation between the presence of pesticides in indoor dust and the biological pesticide contamination of inhabitants.

To conclude, our results allowed the development of the TESTIS project, which has received financial support and is currently on-going. This project will help to fill the gaps in knowledge concerning the impact of prenatal exposure to pesticide on the risk to develop TGCT. This thesis also provided important and original information considering indoor pesticide contamination, the determinants of the agricultural pesticide drift and the factors influencing the dust collection efficiency. In addition to the TESTIS project (on-going), further multidisciplinary studies are under development, based on the results of the thesis.

V.2 Discussion

To address current hypotheses on TGCT etiology, this thesis aimed to develop an epidemiological approach to assess the relationship between prenatal pesticide exposure and risk of TGCT. The specific research objectives of the thesis were: 1/ to identify more precisely gaps in knowledge on environmental and occupational risk factors of TGCT, through the conduct of a literature review; 2/ to develop GIS metrics to assess environmental pesticide exposures more reliably; 3/ to conduct a pilot case-control study to optimize the study design for future implementation in France, and to examine the feasibility of estimating exposures dating back to the 1970's; 4/ to design a case-control study to be conducted in France in accordance with the aim of the thesis and informed by findings from the other objectives of the thesis.

V.2.1 Developing a French nationwide case-control study on risk factors of TGCT

a) Achievement of the thesis and development of the TESTIS study

Overall, by achieving the different specific research objectives of the thesis, we developed the national case-control study (TESTIS) in accordance with our main objectives. This multidisciplinary project has already been awarded grants by two national programs (INCa and INSERM) and will help to fill the gap in knowledge regarding the impact of prenatal pesticide exposure on the TGCT risk. TESTIS will also provide new insight concerning on-going open research questions, e.g. the hypothesis of combined prenatal and postnatal exposures.

The work presented in this thesis was part of a collaborative program entitled "Pesticides and Cancers", developed in 2010 between the Unit of Cancer and Environment at CLB, and the Section of Environment and Radiation at IARC. Niels Erik Skakkebaek, who suggested first the idea of the TDS (Skakkebaek et al. 2001), was consulted in the development of this program and stressed the need for further epidemiological studies to explore the impact of prenatal pesticide exposure on TGCT during adulthood. Our literature review (first objective of the thesis) confirmed the important gap in knowledge concerning the impact of prenatal pesticide exposures

– especially environmental ones – on the TGCT risk. Among the available studies, crude approaches used to estimate pesticide exposures limited the ability to draw firm conclusions. Also, the question of combined effect of prenatal and later life exposures on the risk to develop TGCT remain open.

The second objective of the thesis was designed to improve methods to characterize environmental pesticide exposure assessment, which was a need identified from our literature review. Our initial objective was to test a GIS metric in the French context, based on existing approaches developed in the US, to assess environmental exposure to agricultural pesticide in households from different agricultural areas. However, data used in the development of the US models were not available in France (i.e. pesticide use registry), and data from the literature were too limited to develop a new metric. The following questions remained open: i/ To our knowledge, no standard existed regarding the optimal buffer size to consider in GIS-based studies, and it was unclear if the buffer sizes suggested by Ward et al. (2006) and Gunier et al. (2011) would be applicable to the French context and to all crop types; ii/ Other determinants have been suggested in the literature (e.g. wind directions (Brody et al. 2002; Brody et al. 2004; Chevrier et al. 2014; Pfleeger et al. 2006) and barriers (Brody et al. 2002; De Schampheleire et al. 2009; Ucar and Hall 2001)) but models using these parameters have never been validated; iii/ in absence of existing validation data, the attribution of weight to the different variables in the model would be inaccurate. Thus, we redefined the second objective of the thesis in order to assess the environmental determinants of the indoor contamination by agricultural pesticides, according to three different crop types in the French context. Based on our findings, the GIS metric will be developed in the near future, but will require new samples for its validation. Meanwhile, information on the main environmental determinants of the exposure we identified will be collected in the TESTIS project. By showing the importance of the domestic source of pesticide exposure, the SIGEXPO study also contributes to improve the reliability of the exposure assessment strategy of the TESTIS study.

The pilot study (third objective of the thesis) confirmed our ability to collect information on subjects' exposures and to geocode subjects addresses with sufficient precision back to the 1970's, which is a prerequisite for GIS-based exposure assessment. By testing different

approaches for case and control recruitment and by testing our approach for data collection, the pilot study greatly contributes to optimize the study design of the TESTIS study and the strategy for exposure assessment.

b) Strengths and limitations

The TESTIS study will be the first to assess environmental pesticide exposures using GIS, the overall pesticide exposure assessment on prenatal periods on such a large number of case and controls, and combining prenatal and young-adult exposures. Moreover, our approach to facilitate potential pooled studies analyses helps to address the current limitations related to the lack of power in studies focusing on rare diseases or exposures with low prevalence (e.g. environmental exposures).

However, the TESTIS study may potentially have limitations. Firstly, self-report is the only solution to assess retrospective exposures related to domestic pesticide applications. To be able to identify the active ingredients corresponding to the domestic exposures, we need to combine self-reported information on the type of domestic pesticide use and expert assessment. However, the only existing matrix linking domestic pesticide usage and active ingredient have been developed for the US, and experts will be needed to validate this matrix in the French context. Colt et al. (2004) showed significant association between self-report of several pest treatments used and detection of 15 pesticides in vacuum bag samples from 513 Californian households. Significant correlations suggested that questionnaire-based exposure assessment may be relevant for retrospectively assessed domestic exposure to pesticides. However, only a fraction of the variability of domestic pesticide concentrations was explained by self-reported use of domestic pesticides (r^2= 0.09–0.39). Moreover, recall bias is likely in the case-control design, since the exposure is collected after the diagnosis of the TGCT. In this specific situation, one would be more concerned that recall bias may lead to an inflation of risk estimates, as cases tend to over-report such exposures (Schüz et al. 2003).

The second potential limitation is that several external databases are needed to develop our future GIS metrics. Some have already been identified and cover certain periods of interest (e.g. meteorological information) or should not change over time (e.g. topographic information). However, in regards to information on land use and vegetative barriers, no available database exists before the 1990's, and it will be necessary to construct these data. Remote sensing based on infra-red satellite images as well as photography interpretation based on aerial photography should be an interesting approach (see the TESTIS protocol, part IV.3). However, quality of satellite and aerial images may vary across time and it is not clear how these images can be converted in usable land use information. Thus, further developments are needed in this area.

Finally, some forms of infertility and TGCT may have common origins, considering the TDS hypothesis. Thus, focusing on subjects having a normal sperm production (and excluding men having cryptorchidism) would exclude potential forms of TDS in the controls. However, some forms of infertility may be related to later life exposure, which include pesticides (Bretveld et al. 2007). Thus, by selecting fertile/fecund controls, our control population may have been less exposed to pesticides compared to the general population, which can over-estimate the prevalence of the pesticide exposure among TGCT cases. This bias would appear only when focusing on adulthood exposures, not for our main objective, which is about prenatal exposures. Testing another control group, e.g. from existing cohorts, could complement the TESTIS study to interpret potential associations between TGCT and adulthood exposures.

V.2.2 Studying environmental pesticide exposures

a) Environmental determinants of the indoor pesticide contamination

Results obtained in the SIGEXPO study are in the core of the thesis, and show some intriguing and new findings. To the best of our knowledge, this is the first study to describe the indoor pesticides contamination by such a broad number of pesticides (more than 400), for 239 households. This is also the first study to assess prevailing winds and barriers as environmental determinants of the indoor dust contamination and the first to assess the best buffer size for

different crop maps. Through a grant of the Rhône-Alpes region, Pr. John Nuckols (Colorado University, CO, US) and Dr. Mary Ward (National Cancer Institute, MD, US), both expert in GIS-based pesticide exposure assessment, contributed to this study.

The methods used by Brody et al. (2004) and Chevrier et al. (2014) to implement GIS with wind data only considered the major wind direction, by applying an *a priori* weighting on crops surface in upwind and downwind areas. However, wind direction patterns are changing and these existing approaches cannot integrate information on the variations in the wind direction. With regard to barriers, we did not find any existing approach to integrate these variables in the GIS. The main difficulty was to automatize the detection of barriers, considering that barriers may exist only if hiding the source of the exposures source (i.e. crop area) from the perspective of the targeted households. To solve these technical problem, we developed a new approach (collaborative work with Jeffery Blain, doctoral student in Geography; Elodie Faure, GIS engineer; and Pr. John Nuckols). We first built "contributed areas for pesticide drifts" (CAP) which represent slices of the buffer for the eight major cardinal directions (resulting in eight CAPs). Using the CAP, we first determined ECA to implement data on the prevailing winds in the GIS. Then, we automated the detection of the potential barriers by applying two simple conditions: i/ the barriers had to cross completely the CAP; ii/ the barriers had to be located between the household and a piece of targeted crop map located in the same CAP. By this approach, wind and vegetative barriers have been identified as significant predictors of the exposure, which confirms previous findings and the hypotheses presented above. However, the way prevailing winds and vegetative barriers were operationalized and modeled in the analysis should had an impact on the significance of the findings. Further investigations should be made to confirm our findings and improve approaches to assess these variables in GIS.

b) Indoor contamination of the French households

In 2012, additional funding from the Rhône-Alpes region allowed us to expand the measurements to 416 compounds (including 406 pesticides) for all the dust samples. This was the opportunity to describe more in depth the pesticide contamination of households likely to be highly exposed

(close to agricultural crop maps, during the highest period of pesticide use) or lowly exposed (away from any agricultural fields, outside major period of pesticide use, in the city of Lyon, which has applied a "zero-pesticide" policy since 2008). This was also the opportunity to determine the proportion of the indoor exposure attributable to the outdoor agricultural practices.

In the vast majority of recent environmental studies, exposures to agricultural pesticides have been assessed using GIS, whereas domestic exposures have been neglected. Our results suggested that domestic pesticide could be a major source of contamination, and studies only based on GIS to asses individual exposure to pesticides would lead to bias from exposure misclassification. To our knowledge, this is a unique finding of our study, but it is not surprising since existing studies are focusing on a very limited number of compounds and were not designed to compare the different sources of exposures as we did. Our findings are in line with a previous study from Provost et al. (2007). In this study, increased risks of brain tumors were found among the highest exposed group of vineyard farmers in Gironde (France) and the group that used pesticide indoor to treat plants (OR = 2.16 (IC95% 1.10 – 4.23) vs. OR = 2.24 (IC95% 1.16 – 4.30)), suggesting effects of both occupational and domestic exposures. Overall, our findings suggest that the strategy for pesticide exposure assessment should not only consider occupational and environmental exposure, but also domestic exposure sources. Based on this observation, domestic pesticide exposure assessment has been added to the protocol of the TESTIS study. However, further study is needed to determine how to combine occupational, environmental, and domestic exposures to pesticide in an overall pesticide exposure score, since no information about the relative intensity and duration of these exposures exist.

c) Test of the collection efficiency of the cellulose wipe

Assessment of the wipe efficiency and repeatability was done in a second step, after the sampling phase of the SIGEXPO project. This ancillary project was conducted by Joane Cettier, Masters Student under my supervision. Initially, it was designed to support the findings of the SIGEXPO project. The results we obtained provide interesting and unique information regarding the influence of the dust particles on the physicochemical interaction between the compounds, the

wipe and the extraction solvent. The results (in presence of dust) suggest a good overall collection efficiency (72%) but with a limited repeatability. However, it was difficult to clearly identify the source of the variability, which may be due to extraction process, to the physicochemical properties of the wipe, or to our methodology for spiking the dust and depositing the dust from the aluminum foil to the tile surface. Considering our results in the SIGEXPO study, we assumed that higher variability in measures should have reduced the variability explained by our models. By comparing our results to the studies from Bernard et al. (2008) and Deziel et al. (2011), we found lower collection efficiencies and repeatability in our study. However, it is difficult to compare since we chose real life situations to realize our replicates (environmental pesticide concentration, rough or porous surfaces typical in French households), whereas these former studies used higher concentrations and fewer compounds. Moreover, Deziel et al. (2011) used stainless steel surface as encountered in laboratory settings.

Since dust traps collect dust by passive deposition, there is no impact of the affinity of the compounds for the sampling surface. However, volatilization of pesticides from the dust trap cannot be excluded, and different profile of pesticides has been detected by using the dust trap and the floor wipes. These differences may be related to the different locations in the households (Edwards et al. 1998), but physicochemical properties may also play a role in the interactions between the compounds, the dust, and the dust trap, as demonstrated in our study of the cellulose wipes. However, assessing the collection efficiency and repeatability of the dust trap will require solving some technical issues, including the homogenous passive deposition of organic compounds onto the dust trap replicates.

d) Strengths and limitations

Our findings will help improving pesticide exposure assessment in future epidemiological studies focusing on environmental pesticide exposures. The test of the cellulose wipe also presented original findings by demonstrating the impact of the dust on the pesticide collection efficiency. It provided key elements for future validation studies and would help developing more efficient strategies to monitor indoor exposures based on indoor dust sampling.

The SIGEXPO project has been conducted in the Rhône-Alpes region whereas the TESTIS project will cover the whole France, where local conditions and agricultural practices may vary. The decision to extend the TESTIS study to the whole country was decided after the completion of dust sampling in the SIGEXPO project, and for financial constraints it was not possible to extend the dust sampling to another area. However, the range of different crop types as well as different environmental settings that were considered in the SIGEXPO study will facilitate the extrapolation to the rest of the country, as many different agricultural activities are represented in the Rhône-Alpes region. Moreover, the study by Chevrier et al. (2014) which was done in Brittany confirmed our observations among wind and crop acreage. Replication studies in other French regions or other European countries can be made in order to confirm this finding and adapt the GIS metrics if necessary.

Since the majority of the pesticides were below the 30% detection rate, it was not possible to use classic approaches like Tobit regression or multiple imputations routinely used in similar studies (Lubin et al. 2004). Below 30% of detection rate, these models can be unstable and results over-interpreted (the model will impute the values assuming a normal distribution). Thus, we decide to use RDA – mainly used in the field of ecotoxicology, which consist in a multivariate (multiple dependent variables) and multivariable approach (multiple independent variables). RDA allowed us to study dependent variables having a high proportion of values below the detection limits, and providing the percentage of variability of the dependent variables explained by the independent variables. However, RDA obscured the difference observed between individual pesticides, which may be important, as shown in the Tobit analyses of four pesticides (part III.4.3).

Our study participants were not representative of the general population, and the study was not initially designed to assess detailed pesticide exposure in the general population. By sampling households close to crop maps and during periods of pesticide applications (Zone 1 – 3), we aimed to describe a group with high levels of environmental pesticide exposure. Conversely, the 4th zone (urban, sampled outside main period of pesticide utilization) was chosen to represent the background level in the general population. The differences of contamination between these two extremes give an idea of the pesticide exposure level attributable to environmental pesticide exposures. However, while a recent US study has highlighted the temporal variability in terms of indoor dust pesticide contaminations (Deziel et al. 2013; Obendorf et al. 2006), our study was based on a unique sample per households. Repeated sampling should be made in order to interpret the seasonal variation in the different zones samples.

V.3 Research perspectives

a) The TESTIS project

The TESTIS project has been awarded grants by the INCa and the INSERM at the end of 2013, and the recruitment is planned to start in October 2014. The storage of biological samples and the geocoding of the study subjects will allow participation in future consortiums or the realization of ancillary project to validate future hypotheses. During a two-week stay at the NCI, I also tried to harmonize questionnaire items of interest and the protocol for genetic analyses with existing studies conducted at the NCI to facilitate further pooled analyses. Lastly, measurements of pesticides or other organic compounds in plasma of the TESTIS participants may be used to validate or replicate different approaches for exposure assessments (e.g. questionnaire, GIS metrics).

The approach and the tools developed for the TESTIS project may be applicable to other projects. Indeed, based on the controls of the TESTIS project, a cross-sectional study has been planned by the CECOS network. The PESTIMAL project aims to identify potential environmental exposures associated to altered sperm characteristics. A grant application for this project has been submitted to the *Fondation de France* (summer 2014).

b) The SIGEXPOSOME project

A future GIS metric should be developed based on the determinants of the exposure that we identified in the SIGEXPO study. However, another set of data would be needed to validate the metric before being routinely used and further additional research questions have arisen, based on the findings presented in this thesis. Other variables having potential impact on the pesticide drift should be tested as determinants of the exposure in future analyses (e.g. rain or soil type), in order to improve the GIS metric. Moreover, it is still not clear to what extend indoor dust contamination is predictive of personal biological contamination. This link needs to be established to support potential association between agricultural pesticide exposures assessed

through our GIS metric and increased risk of specific diseases. This information is also needed to assess whether a potential risk for the general population exists based on our indoor dust measurements.

To fill these gaps in knowledge, a new project call “SIGEXPOSOME” is under development. This project will start in December, 2014. The SIGEXPOSOME project will include repeated indoor dust and biological sampling (blood and urine) among 200 volunteers (50 pesticides applicators and 150 volunteers from the general population living close to vineyards). The main objective of this project is to assess the correlation of the agricultural pesticide exposure in biological samples with the exposure level assessed through GIS and indoor dust measurements. This project will also contribute to identify biomarkers of effect and provide information on the relative intensity of exposure attributable to occupational and environmental exposures.

This SIGEXPO / SIGEXPOSOME study designs may serve as basis for other teams willing to replicate our findings and extrapolate determinants of the agricultural pesticide exposure in other regions.

V.4 Conclusion

Based on the approach developed in this thesis, the TESTIS study will help to fill gaps in knowledge concerning TGCT etiology. The study has been funded and recruitment has been started. Few studies have investigated prenatal exposure related to TGCT risk, and this is the first time that prenatal environmental exposure to pesticides, as well as potential combined exposures during the prenatal and adulthood time period, will be explored. While TGCT is a rare disease having a good prognosis, our findings will also provide new insight concerning the origins of male infertility and congenital sexual malformation (considering the TDS hypothesis), which concern many more people.

This thesis also provides new and important findings in the field of the pesticide exposure assessment. For the first time, we convincingly show the substantial proportion of domestic pesticides – compared to agricultural pesticides – in the indoor dust pesticide contamination, based on a large number of samples and a large number of pesticides screened. Also, a unique finding of this work was to show the differences in terms of pesticides drifts depending to different crop types, and to define the prevailing winds and the vegetative barrier as determinants of the agricultural pesticide level in house dust. These findings were reinforced by our validation studies showing the overall good pesticide collection efficiency of the cellulose wipe used for indoor dust samples. The approach we developed to assess the impact of the prevailing winds and the barriers should be of interest for future GIS-based study or GIS metrics.

This thesis will serve as basis for on-going and future collaborative research between CLB and IARC and was enclosed in the collaborative program on “Pesticide and Cancer”, supported by the LYric (the integrated cancer research site of Lyon). Our study meets the research priorities identified by the INSERM collective expertise 'Cancer and the Environment’ (INSERM 2008) and responds to the measures of the priorities identified by French National Cancer Plan 2014-2019 and the French National Health and Environment Plan 2009-2014 (PNSE). A second project, deriving from the SIGEXPO project, is currently under development. Other multidisciplinary ancillary projects will be developed based on these projects in the future years.

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ANNEXS

Annex 1: Curriculum vitae

Education and training

Feb. 2011 – Dec. 2014: Ph.D in Public Health, University of Lyon, France: "Testicular germ cell tumors: Assessing the impact of occupational and environmental exposure to pesticides"
(Doctoral grant from the Rhône-Alpes region, France)

- *Section Environment and Radiation, International Agency for Research on Cancer*
- *Unité cancer et environnement, Centre Léon Bérard*

Aug. 2013: Training in "logistic regressions" (teacher: Pr. Stanley Lemeshow), Erasmus Summer School, University of Erasmus, Rotterdam, The Netherland.

Jun. 2013: Training in "scientific writing in English" (teacher: Dr. Margaret Haugh; Medicom Consult'),

Sept. 2008 – Jun. 2010: M.Ph "engineering for health and environment", specialty "research methodology in health and environment", University of Grenoble, France.

Sept. 2002 – Jun. 2008: State Diploma of Midwifery, Faculty of Medicine of Grenoble, University of Grenoble, France.

Professional experience

Since Sept. 2010: Teaching in epidemiology and research methodology (~150h in total): up to the master level (University of Lyon and Grenoble); teacher assistant at the IARC Summer School (2011).

Feb. 2014: Mobility at the National Cancer Institute (MD, US)

- *Occupational Epidemiology Branch (contact: Dr. Mary Ward)*
- *Hormonal and Reproductive Epidemiology Branch (contact: Dr. Katherine McGlynn)*

2012 – 2013: Workgroup on "reproductive toxicity in professional environments", French National Institute of Research and Safety (head: Dr. Dominique Lafon)

Jan. – jun. 2010: Internship (master): "Occupational chemical exposures and premature ovarian failure: development of a study protocol"

- *Equipe EPSP-TIMC, UMR CNRS 5525, Université Joseph Fourier (UJF) - Grenoble1*
- *Service de Procréation Médicalement Assistée du CHU de Grenoble*

Jun. 2008-oct. 2010: Midwife, Regional Hospital of Chambery, France

Expertise and skills

Fields (environmental epidemiology, pesticide exposure assessment, geographic information systems, embryology and genital development, Testicular germ cell tumors, male and female infertility)
Project design (3 projects fully designed, participation to 2 other projects – all funded)
Grant writing (participation to the design of grant applications: ~1,200 K€ obtain since 2011 from regional and national programs)
Computer skills (Microsoft Office Suit (advanced); SAS, Stata and R (basic)
Languages: French (maternal), English (advanced), German (basic)

Annex 2: List of publications & communications

Publications

- **Related to the present thesis**

Béranger R, *Billoir E, Nuckols JR, Faure E, Blain J, Chasles V, Schüz J, Fervers B. Environmental determinants of the indoor exposure to agricultural pesticides. (Under finalization) To be submitted to Environmental Health Perspective.*

Béranger R*, Billoir E, Nuckols JR, Blain J, Combourieu B, Philip T, Schüz J, Fervers B. Agricultural and domestic pesticides in house dust from different agricultural areas in France. Submitted to Environmental Health Perspective.*

Cettier J, Bayle ML, **Béranger R**, Billoir E, Nuckols JR, Combourieu B, Fervers B. Efficiency of wipe sampling on hard surfaces for pesticides and PCBs residues in house dust. Science of the Total Environment **(in press)**.

Béranger R, Pérol O, Bujan L, Faure E, Blain J, Le Cornet C, Flechon A, Charbotel B, Philip T, Schüz J, Fervers B. Studying the impact of early life exposures to pesticides on the risk of testicular germ cell tumors during adulthood (TESTIS project): study protocol. BMC Cancer. 2014; 14:563.

Béranger R, Blain J, Baudinet C, Faure E, Fléchon A, Boyle H, Chasles V, Charbotel B, Schuz J, Fervers B. Tumeurs germinales du testicule et expositions précoces aux pesticides : étude pilote TESTEPERA. Bulletin du cancer. 2014 Mar;101(3):225-35.

Béranger R, Le Cornet C, Schüz J, Fervers B. Occupational and environmental exposures associated to testicular germ cell tumours: systematic review of prenatal and life-long exposures. PLoS One 2013 Oct 14;10(8): e77130.

- **Other publications**

Le Cornet C, Fervers B, ***Béranger R****, Olsson A, Oksbjerg Dalton S, Hansen J, Feychting M, Pukkala E, Tynes T, Kauppinen T, Kristjansson V, Uuksulainen S, Wiebert P, Woldbaek T, Skakkebæk NE, Schüz J. Testicular cancer and parental occupational exposure to pesticides: a register-based case-control study in 4 Nordic countries. (Under finalization) To be submitted to international journal of cancer.*

Le Cornet C, Lortet-Tieulent J, Forman D, **Béranger R**, Flechon A, Fervers B, Schüz J, Bray F. Testicular cancer incidence to rise by 25% by 2025 in Europe? Model-based predictions in 40 countries using population-based registry data. European journal of Cancer. 2014 Mar;50(4):831-9.

Béranger R, Hoffman P, Christin-maitre S, Bonneterre V. Occupational exposure to chemicals as a possible etiology in premature ovarian failure : a critical analysis of the literature. Reprod toxicol. 2012 Jun;33(3):269-79

Oral Communications

- **As invited speaker:**

Béranger R, Hoffman P, Christin-maitre S, Bonneterre V. Potential impact of endocrine disruptors on premature ovarian failure. [Oral presentation] Workshop "Reproduction and Toxicant"; April 8-9th, 2014; Catanzaro, Italy.

Béranger R, Hoffman P, Christin-maitre S, Bonneterre V. Impact des facteurs environnementaux sur l'Insuffisance Ovarienne Précoce. [Oral presentation] Journée Environnement et Fertilité Merck-Serono ; 17 novembre 2012 ; Paris, France.

- **On abstract submission:**

Béranger R, Billoir E, Nuckols J, Blain J, Bayle ML, Schüz J, Combourieu B, Fervers B. SIGEXPO project: pesticides exposure level in the French context: burden of environmental exposures and domestic habits. Abstracts of the 2013 Conference of the International Society of Environmental Epidemiology (ISEE), the International Society of Exposure Science (ISES), and the International Society of Indoor Air Quality and Climate (ISIAQ), August 19–23, 2013, Basel, Switzerland. 2013. Environ Health Perspect; http://dx.doi.org/10.1289/ehp.ehbasel13.

Béranger R, Blain J, Billoir E, Chasles V, Schüz J, Combourieu B, Fervers B. SIGEXPO: Geographic Information System validation using indoor dust samples. [Oral presentation] 8th Cancer Scientific Forum of the Cancéropôle CLARA; March 21-22, 2013; Lyon, France.

Béranger R, Blain J, Saout K, Fervers B, Schüz J, Combourieu B, Chasles V. Geographic Information Systems in pesticides exposures prediction: validation by dust sample. [Oral presentation] X 2012 congress, 7th International Conference on the Science of Exposure Assessment; 2-5 july 2012; Edinburgh; UK.

Béranger R, Hoffman P, Christin-maitre S, Bonneterre V. Les expositions chimiques professionnelles comme étiologie possible des insuffisances ovariennes précoces. [Oral presentation] 32e congrès national de médecine et santé au travail ; 5-8 juin 2012 ; Clermont-Ferrand ; France

Béranger R, Le Cornet C, Fléchon A, Boyle H, Schüz J, Fervers B. Environmental and occupational exposures in testicular germ cell tumours. [Oral presentation] 7th Cancer Scientific Forum of the Cancéropôle CLARA; March 20-21, 2012; Lyon, France.

- **Internal seminar**

Béranger R. The use of Geographic Information Systems for pesticide exposure assessment. [oral presentation] ECSpertise seminar, IARC, Lyon, France, May 28th, 2014.

Béranger R, Pérol O, Fervers B, Schüz J. TESTIS Project. [Oral presentation] Internal seminar, National Cancer Institute, MD, US, February 12th, 2014.

Béranger R, Fervers B, Schüz J. SIGEXPO : indoor dus sampling to develop a metric for pesticide exposure assessment in France. [Oral presentation] Internal seminar, National Cancer Institute, MD, US, February 6th, 2014.

Posters

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Résumé de la thèse

Les tumeurs germinales du testicule (TGCT) sont la forme de cancer la plus fréquente chez les hommes jeunes (15-39 ans). Un rôle de l'environnement au moment de la période prénatale est suspecté, mais aucune étiologie claire ne semble émerger. Cette thèse avait pour but de développer une nouvelle approche épidémiologique pour étudier l'impact des expositions prénatales aux pesticides sur le risque de TGCT. Par une revue de la littérature, nous avons tout d'abord montré le manque d'études sur les expositions prénatales et le besoin de méthodes fiables pour évaluer l'exposition environnementale aux pesticides. Ensuite, par une campagne de mesures domestiques dans 239 foyers, nous avons identifié les déterminants environnementaux de l'exposition aux pesticides agricoles. La surface des cultures dans un rayon de 500m (vergers) ou 1000m (céréales/vignes), le vent et les barrières végétales ont été identifiés comme déterminants de l'exposition. La bonne efficacité de notre lingette en cellulose a été testée en laboratoire. Nos résultats montrent également l'importance des utilisations domestiques de pesticides sur la contamination des foyers. Enfin, à travers une étude cas-témoins pilote, nous avons confirmé notre capacité à recruter des sujets et leurs mères, ainsi que les informations requises pour évaluer les expositions jusque dans les années 70. Pour conclure, nos résultats ont permis le développement d'une étude cas-témoins nationale (projet TESTIS) pour étudier l'impact des expositions prénatales aux pesticides sur le risque de TGCT. Ce projet a été financé et est en cours de réalisation. Cette thèse sert également de base à plusieurs autres projets multidisciplinaires.

Mots clés : Tumeurs germinales du testicule ; Pesticides ; Système d'information géographique ; Expositions environnementales ; Epidémiologie ; Revue de la littérature ; Etude de validation.

Abstract of the thesis

Testicular germ cell tumors (TGCT) are the most common cancers in men aged 15–39 years. Environmental exposures occurring in the prenatal period are suspected to play a role, but no clear associations with TGCT risk are known. This thesis aimed to develop an epidemiological approach to study the impact of prenatal exposures to pesticides on the TGCT risk. First, through a systematic literature review, we identified a gap in knowledge regarding prenatal exposures, as well as the need for more reliable assessment of environmental pesticide exposures. Second, through a survey of indoor dust sampling in 239 households, we identified the environmental determinants of agricultural pesticide exposure to develop a metric to assess environmental pesticide exposures using a geographical information system. Crop acreage within 500m (orchards) or 1000m (cereals/vineyards), wind, and vegetative barriers were identified as determinants of the indoor contamination. The overall good efficiency of our cellulose wipe was assessed through laboratory experiments. Our results also suggested domestic pesticide use as a major source of households' pesticide exposure. Third, through a case-control pilot study we tested different approach to recruit young men and their mothers, and we confirmed our ability to collect information about their exposures, and to map precisely their addresses until the 1970's. Our findings lead to the development of a national case-control study (TESTIS project) aiming to assess the impact of prenatal pesticides exposures on the TGCT risk. This project has been funded and is currently on-going. Our research also serves as basis for further multidisciplinary projects.

Keywords: Testicular Neoplasms; Pesticides; Geographic information systems; Environmental exposures; Epidemiology; Literature review; Validation study.